United States
Department of
Agriculture

**Agricultural
Research
Service**

December 1985

1984 Stone Fruit Tree Decline Workshop Proceedings

United States
Department of
Agriculture

**Agricultural
Research
Service**

1984 Stone Fruit Tree Decline Workshop Proceedings

Charles L. Wilson and Ralph Scorza
Workshop Coordinators

Proceedings of a workshop held
October 30-November 1, 1984
at the Appalachian Fruit Research Station
Kearneysville, WV

CONTENTS

The papers, including the figures, references, and tables, presented here are reproduced essentially as provided by the individual authors. Queries regarding them should be referred to authors of specific papers. The views expressed by the workshop participants are their own and do not necessarily represent the views of the U.S. Department of Agriculture.

As the papers were prepared in reproducible form by the individual authors to expedite the publishing of the proceedings, there is some variation in type and format; however, such variations should not distract the attention of the reader.

Copies of this report can be purchased from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

ARS has no additional copies for free distribution.

INTRODUCTION

Charles L. Wilson and Ralph Scorza
USDA/ARS Appalachian Fruit Research Station
Kearneysville, WV 25430

The first Stone Fruit Tree Decline Workshop held at Michigan State University in 1982 brought together scientists from different disciplines and geographical areas in the USA and Canada to discuss reduced stone fruit tree productivity and longevity. A number of factors were considered including cultural practices, nutrition, cold temperatures, mechanical harvesting devices, wound compartmentalization, pathogenic fungi and bacteria, nematodes, mycoplasmas, and viruses. The attendees had the opportunity to gain insight into new areas of research. It was hoped that cooperative efforts would develop from contacts made at our initial meeting. This volume of the Proceedings of the second Stone Fruit Tree Decline Workshop attests to the success of that first meeting. A number of cooperative efforts are now underway in the search for causes and solutions for this difficult problem. It is evident that Stone Fruit Tree Decline is not the result of a single major factor but a complex interplay of factors acting in yet to be understood sequences. These factors and their interactions may differ with locality. Tree decline in one region may have completely different causes than in another. A variety of proposed causes for tree decline were presented at this workshop. Often the primary cause was not clear. Conventional thinking using simple cause and effect relationships can make it difficult to rationalize the decline problem. Particularly when we assume that each disease has one cause. In Stone Fruit Tree Decline it sometimes appears that we have one disease with many causes. Can a tree respond with the same disease to different causal agents? Can there be multiple and simultaneous causes of the same disease? If so, the number, severity, and timing of insults to the tree may be more important than the nature of the insults themselves.

Such questions led to the perception of a commonality binding the various aspects of tree decline syndrome. It was the tree itself. When discussions focused on the tree a new rationale emerged. It appeared that different insults (disease, cold damage, mechanical damage) can elicit similar responses by the tree. In these cases the tree walls off or contains the damaged tissue (an energy requiring process) and new regenerative cells are formed which continue the growth process (requiring additional energy). Life cannot continue without growth. Growth cannot occur unless plants exceed their compensation point (i.e. manufacture more food than they consume). In its simplest terms trees decline when their energy requirements to defend themselves exceed their energy requirements for other living processes

The tree's energy reserves then become the major limiting factor for its defense system. Hypotheses concerning tree energy reserves and mechanisms of response to wounding and disease invasion were among the topics of lively discussion during this second workshop. If tree energy reserves prove to be an important factor in the decline syndrome, cultural practices which alleviate the stress of unbalanced energy demands and the development of genotypes with a greater energy storage capacity along with a more effective compartmentalization capability will be critical to preventing tree decline.

Approaching tree decline from varied points of view, from different disciplines, through field and lab work is a task that no one person or group can accomplish alone. Communication between researchers is essential. The Stone Fruit Decline

Workshops are meant to foster such communication. In the future, workshops should seek additional input from researchers working with other tree crops including forests trees, citrus and tropical fruits. We hope that new cooperative efforts will develop as a result of this second Stone Fruit Tree Decline Workshop and look forward to progress in increasing stone fruit tree productivity and longevity.

We take this opportunity to thank all those at the Appalachian Fruit Research Station who gave so willingly of their time to perform those tasks necessary for conducting this workshop. We would particularly like to thank: John Cordts; Jerry Franklin; Dixie Gaynor; Lenard Gilreath; Polly Hess; Gary Lightner; Janice McMahan; Brian Otto; Joy Silvius; Wanda Stouffer; Claire Stuart; and Wayne Zook. A video recording of the workshop was made and copies are available by contacting Gary Lightner at the Appalachian Fruit Research Station.

245

Host Response to Cytospora Canker and the Possible Role of Carbohydrate Reserves in the Peach Decline Syndrome

Michael Wisniewski and Charles Wilson
USDA/ARS, Appalachian Fruit Research Station
Kearneysville, West Virginia 25430

Introduction

Shigo and Wilson (1982) have suggested that peach trees have a poor ability to compartmentalize injury and decay and that the cumulative effects of increasing energy demands for defense mechanisms as wounds accumulate may play an important role in the decline syndrome. In addition, Wilson, et al. (1984) have demonstrated that pruning technique may affect the degree of dieback and susceptibility to Cytospora infection. The question has been raised whether or not qualities favoring tree longevity have been accidentally bypassed in the selection process for fruit qualities Wilson, et al. (1982).

In the following report we would like to present information on structural features and physiological responses relevant to the process of compartmentalization and then speculate on the long term impact of these processes on carbohydrate reserves, their allocation, and their possible role in the decline syndrome. Presentation of the first two topics will be brief since the results have been published previously or are in press. Instead, we will spend most of our time speculating on the latter topic.

Anatomical Response to Infection

A developmental study was conducted of the infection process with 1-year old stems of mature, 'Loring peach' trees that were inoculated with mycelial plugs of Cytospora leucostoma. The infection process was compared to cytological events that occurred in non-inoculated, wounded samples. Infection was followed over an eleven-month period. Details of this study are forthcoming (Wisniewski, et al., In Press).

A few salient points are as follows:

a.) Both the timing and intensity of necrophylactic periderm (NP) formation was affected by the presence of C. leucostoma. Larger areas of initial necrosis were induced by the pathogen without inducing NP formation. NP formation was delayed by 1-2 weeks and when present was incomplete in inoculated samples.

b.) Hyphae of C. leucostoma proliferated in necrotic tissue forming dense mycelial wedges that were able to penetrate (presumably, by physical pressure and enzymolysis) necrophylactic periderms formed within the host. This allowed the constant spread of the pathogen into living tissue and gradual enlargement of the canker.

c.) A marked lack of differentiation of callus tissue occurred in inoculated samples. Whereas the callus tissue in non-inoculated samples quickly differentiated into typical xylem and inner and outer bark, the callus tissue of inoculated samples was slow to differentiate and remained parenchymatous as long as five months following inoculation.

d.) Inoculated samples exhibited a much stronger gumming response compared to non-inoculated, wounded samples. Although a zone of gum ducts was initiated in both cases, gum ducts in inoculated samples were larger and produced more gum over a longer period of time.

The role of the gumming response in peach trees is problematic. Production of copious amounts of gum (gummosis) may represent a general response to tissue injury, (Boothby, 1983). However, despite the fundamental nature of the gumming response, its proven effectiveness as a protective barrier or agent has yet to be documented (Grosclaude, 1966). On the contrary, many adverse effects may result from gummosis. Production of copious gum requires extensive metabolic activity and represents a substantial loss of carbohydrates (Boothby, 1983). Strong gumming responses also induce xylem dysfunction and concomitant water stress (Hampson and Sinclair, 1973) which in turn may have the short term effect of increasing susceptibility to Cytospora (Bertrand, et al., 1976). In addition, in our study, growth of Cytospora was not inhibited by the presence of gum and Gairola and Powell (1971) have reported that Cytospora possesses xylotic enzymes which enable the fungus to metabolize gum.

Long-term effects of gummosis, especially when induced by canker organisms such as Cytospora, on translocation of carbohydrates and over-all levels of carbohydrate reserves have not been studied. Yet it is plausible to suggest, as have Shigo and Wilson (1982) that production of copious amounts of gum, seemingly without much benefit, may represent a high cost expenditure in terms of available resources. This situation may lead to poor growth and perhaps, on a long term basis, a depletion of reserves. This energy-stress may play a role in peach decline by making the tree more susceptible to the adverse effects of a number of biotic and/or abiotic factors. Resource allocation and carbohydrate turnover are discussed in more detail below.

Evidence from our studies further suggest that increased gum response may inhibit the differentiation of callus tissue by disturbing or delaying balanced metabolic processes that occur during the normal formation of xylem and phloem (Wisniewski, et al., In Press). The persisting parenchymatous, callus tissue in inoculated samples appears to be very susceptible to continued ingress by the pathogen. Ingress of the pathogen into healthy tissue apparently stimulates a continued gumming response which in turn has the effect of perpetuating tissue that is very susceptible to invasion by the encroaching fungus.

It is interesting to note, in regard to gummosis, that Okie and Reilly (1983) have demonstrated that several exotic lines of peach trees exhibited high levels of resistance to gummosis induced by Botryosphaeria dothidea. This was despite their ability to re-isolate the causal organism from a number of wounds. This material should prove to be an excellent source for studying the fundamental differences between susceptible and resistant responses.

Interaction Between Cytospora leucostoma and Host-Phenolic Compounds

The presence of phenolic compounds in plant tissues has been shown to have a significant influence on disease development in many host-parasite systems (Horsfall and Cowling, 1980; Kuc and Shain, 1977). In trees, phenol enriched "reaction zones" are generally thought to act as chemical barriers that prevent or retard pathogen movement within the tree (Shortle, 1979a&b; Shain, 1979 and 1967).

We conducted an examination of phenolic response in dormant peach trees of 'Loring' and 'Sunhigh' (Wisniewski, et al., 1984, Wisniewski, 1983). Dormant trees were selected in an attempt to simulate conditions in early spring when temperatures are warm enough for the growth of the pathogen, but trees are still relatively dormant. This period has been cited as a critical one for the development of new infections (Rosenberger, 1982; Scorza, 1982; Wensley, 1970).

In early February, 1982, two branches 6-10 cm apart were pruned from each tree, leaving short stubs (1 cm). One stub was inoculated with an agar plug containing mycelium of C. leucostoma while the other stub received an application of sterile agar. These short stubs and adjacent main tissues were sampled at 5, 7 and 9 weeks and analyzed for total phenolics. Characterization of the zones of tissue sampled is presented in Fig. 1. In addition several long stubs (8-10 cm) were inoculated and examined for patterns of phenolic response.

Figure 2 and Table 1 summarize the results of this study. Figure 2 demonstrates that both treatment and distance from the point of inoculation interact through time to influence the amount of extractable phenolics present (3-way ANOVA, p 0.01). There was no significant difference between cultivars.

Examination of both bark and wood tissues of the main stem (Table 1) indicated that maximum phenolic response occurred in the branch collar region (zones 1, 4) in response to inoculation. Additional experiments have documented a pattern of phenolic levels associated with morphologically distinct zones on long peach twigs infected with C. leucostoma (Wisniewski, 1983). Preliminary observations indicated depleted values near the healthy and infected tissue, and intermediate values behind the peak (Fig. 3).

The compartmentalization of wounds in peach trees is important in resistance to Cytospora (Shigo and Wilson, 1982) and the presence of phenolic enriched "reaction zones" are thought to act as a chemical barrier. In our study, the presence of C. leucostoma played a significant role, over and above the wounding response, in establishing levels of host-phenolic compounds. The strongest phenolic response occurred in the branch collar. Similar responses have been reported in red maple branch stubs (Green, et al., 1981) and black walnut (Armstrong, et al., 1981). The increased levels of phenols, however, seemed to function only as a temporary barrier that eventually is depleted. Levels of phenol in host tissues seemed to increase in advance of visible infection.

Effectiveness of the compartmentalization processes can be augmented or decreased by cultural practices such as pruning (Wilson, et al., 1984) and also by competition for resources within a tree (Wisniewski, 1983). Whether or not phenolic response is augmented by cultural practices such as proper pruning or the maintenance of vigorous trees has yet to be determined.

Effect of Crop Load on Growth and Susceptibility to Cytospora in Peach Trees

Growth in trees is related to the overall supply of resources and to the competition between its parts for those resources. In general, resources are allocated to different portions of a plant on the basis of their strength as a sink (Wareing and Patrick, 1975). By far the strongest sink for carbohydrates and water in bearing peach trees is developing fruit. Chalmers and van den Ende (1975) have reported that peach trees undergo an 80% reduction in the rate of vegetative growth within three years after the initiation of fruit bearing, at which time the rate levels off.

Stassen et al. (1982) have reported that 80% of the total sugar content of a tree is tied up in the fruit at harvest time and that 73% of the available starch reserves are consumed from dormancy up to and including bud-break. These authors have stressed that the post-harvest period is very important for accumulation of carbohydrate reserves and for this reason note the critical importance of the maintenance of healthy, mature leaves for as long as possible into the autumn.

The above mentioned reports strongly suggest that in peach trees, competition for resources can be strong and that levels of non-structural carbohydrates (current and reserve) can be dramatically affected by resource utilization during vegetative and reproductive growth, and also by mechanisms utilized in defense and repair. Shigo and Wilson (1982) have noted the need for concern about the long term energy costs for maintaining mechanisms of compartmentalization for wounds and decay in Elberta peach trees. Allocation of resources to defense and repair in general has also been recently reviewed (Mitra and Bhatia, 1982; McLaughlin and Shriner, 1980). In addition, Houston (1967) has pointed out that, on a long term basis, competition for limited resources may take the form of an overall weakening or loss of vigor of an organism, as occurs with the decline of hardwoods. A similar relationship between reserves and decline has been suggested by Carroll et al. (1983). The relevance of these concepts to the problem of peach decline or short-life should be examined.

It is known that the amount of assimilate available for new growth in mature 'Elberta' peach trees is inversely related to yield (Proebsting, 1958). However, the effects of bearing on other parameters in peach trees, such as mature leaf size, levels of total, non-structural carbohydrates (TNC) and defense and repair mechanisms have not been examined.

In the last section of this paper we would like to present preliminary data from an experiment in which we examined the effect of crop load on growth and susceptibility to Cytospora in 'Loring' peach trees by comparing a number of indicators of growth, patterns of TNC accumulation, and response to Cytospora inoculations in fruiting and deblossomed trees. Details of the experimental protocol and results have been previously presented (Wisniewski, 1983).

Results of the experiment indicated that in general crop load of full bearing 'Loring' peach trees (average yield = 102.27 kg $\pm$ 10.4) had an significant effect on a number of parameters of growth, resistance to Cytospora, and a questionable effect on accumulation of TNC.

Patterns of leaf development were significantly different (Fig. 4) (2-way ANOVA, p .001) and the mean area (cm^2) of mature leaves from the mid-canopy was significantly greater in deblossomed trees (ANOVA, p .001). In addition, patterns of diameter increase in the trunk of fruiting and deblossomed trees (Fig. 5) were significantly different (2-way ANOVA, p .01), with deblossomed trees having a greater total increase (ANOVA, p .01). Proportional increase in diameter of one-year-old twigs (Fig. 6) demonstrated the same relationship (ANOVA, p .001).

Levels of TNC in twigs of deblossomed trees and bearing twigs of fruiting trees fluctuated and were difficult to interpret (Fig. 7). The patterns of levels of TNC, however, were significantly different in the two treatments (2-way ANOVA, p .025). Deblossomed trees attained a higher level of TNC and ended the growing season with a significantly higher level of resources (ANOVA, p .05).

Differences in the growth parameters of fruiting and deblossomed trees were also reflected in differences in susceptibility to Cytospora (Fig. 8). Patterns of canker enlongation were significantly different (2-way ANOVA, p .025) and mean canker size was significantly greater in fruiting trees at the end of October (ANOVA, p .005). This indicated that some measure of at least short term resistance was present in deblossomed trees.

The differences observed between fruiting and deblossomed trees with respect to parameters of growth can be explained by differences in resource allocation. Without developing fruit acting as a major sink (Chalmers and van den Ende, 1975), carbohydrates were probably more available for vegetative growth. Inverse relationships between crop load and assimilate available for trunk growth have been documented in both apple (Maggs, 1963; Avery, 1969, 1970; Mochizuki, 1962; Rogers and Booth, 1964) and peach (Proebsting, 1958) trees.

Avery (1975a) has also reviewed several studies on apple trees which have documented that total amounts of foliage formed as well as individual mature leaf size are smaller in fruiting trees than deblossomed trees. However, it is important to note that rates of photosynthesis and assimilation are much higher in fruiting trees than in deblossomed trees, apparently due to the strong sink of the developing fruit (Avery, 1975b). Similar effects on shoot growth have also been reported (Barlow, 1975), noting that other factors beside crop load, such as pruning, fertilization, water, and light, will also play a role in determining amounts of growth. On the other hand, beneficial effects of reduced crop loads on vegetative growth may also show up in subsequent years rather than within the current season (Barlow, 1975; Avery, 1975a).

Although the estimated levels of total resources fluctuated considerably, the general pattern followed patterns documented for apple trees (Priestley, 1970). Our observations indicated decreasing levels of carbohydrates during bud-break followed by increasing levels as developing leaves began to export photosynthate, then decreasing levels as resources were consumed during current season growth followed by rising levels as the dormant season approached. It is suggested that the fluctuations observed may have been due to peak demands for assimilate during stages of rapid fruit and/or vegetative growth. Establishment of higher overall levels of resources in deblossomed trees also agrees with reports by Priestley (1970) and Avery (1975b) for apple trees.

Regarding the measured resistance to Cytospora in deblossomed trees, it is important to note that this may have been only a short term phenomenon. In addition, it is difficult to attribute the resistance response to any specific condition(s) within the deblossomed trees. Most probably, a number of factors (as discussed below) interacted to produce the measured resistance. This is the first report attempting to associate resistance within a variety to measurable effects of crop load.

Wensley (1966) has reported a correlation between vigorous varieties and canker resistance and the results of this study may be caused by a greater growth potential in deblossomed trees. Bertrand and English (1976) have also reported an association between tree vigor and resistance in French prune trees (Prunus domestica).

Bertrand et al. (1976) have reported an association between moisture stress and short-term resistance in prune trees. Although water potential was not monitored in the present study, Chalmers and Wilson (1978) have reported that expansion growth of limbs is limited by low water potential due to water demands of the fruit

during stages of rapid increase in fresh weight. Hence, when fruit are competing actively for assimilate their water status is favorable for growth, and when the demand of the fruit for assimilate is low the water status of the tree improves and expansion growth of the limbs is possible. This phenomenon may also have played a role in the resistance observed in deblossomed trees.

As mentioned above, levels of available resources play an important role in establishing the efficacy of defense and repair mechanisms, especially on a long term basis. Although it is doubtful that the estimate of TNC measured in this study reflects whole tree status, it is plausible to suggest that levels of available TNC may have played a role in the resistance response. It appears that energy resources in peach trees are somewhat limited (Stassen et al., 1982). If competition is strong for limited available resources it is possible that defense and repair mechanisms may be impaired to some degree by competing demands of developing fruit. Direct mechanisms of resistance (e.g., production of phenolic constituents, activation of compartmentalization processes) as well as indirect mechanisms (e.g., increased growth to facilitate wound closure) may have been operating at a higher level or faster rate in deblossomed trees. In addition, adverse conditions of limited available resources may have been augmented by increased rates of respiration and loss of carbohydrates via gum production in diseased trees.

Although much of the above discussion is speculative, it underscores the need for long term studies of carbohydrate turnover, resource allocation, and effects of many years of bearing on tree vigor. Little information exists on resource allocation for defense and repair in peach trees and its relation to reproductive effort and levels of resources (current and reserve).

Efficient and effective mechanisms of defense and repair are basic features needed within any cultivated crop. A better understanding is needed of how these mechanisms are affected by such things as intensive bearing and pruning over long periods of time. Competition for limited available resources, and decreases in energy generating and storing tissues due to ineffective compartmentalization of wounds and infection, may play a role in peach decline or short life.

Literature Cited

Armstrong, J. E., A. L. Shigo, D. T. Funk, E. A. McGinness, Jr., and D. E. Smith. 1981. A macroscopic and microscopic study of compartmentalization and wound closure after mechanical wounding of black walnut trees. Wood Fiber 13:275-291.

Avery, D. J. 1975a. Reduction in growth increments by crop competition. In: Climate and the Orchard. ed. H. C. Pereira. Res. Rev. 5. Commonwealth Agricultural Bureaux. pp. 110-112.

_____. 1975b. Effects of fruits on photosynthetic ability. In: Climate and the Orchard. ed. H. C. Pereira. Res. Rev. 5. Commonwealth Agricultural Bureaux. pp.110-112.

_____. 1970. Effects of fruiting on the growth of apple trees on four rootstock varieties. New Phytol. 69:19-30.

_____. 1969. Comparison of fruiting and deblossomed maiden apple trees, and on non-fruiting trees on a dwarfing and invigorating rootstock. New Phytol. 68:323-326.

Barlow, H. W. B. 1975. Effects of cropping on the growth of apple trees. In: Climate and the Orchard. ed. H. C. Pereira. Res. Rev. 5. Commonwealth Agricultural Bureaux. pp.98-102.

Bertrand, P. F. and H. English. 1976. Virulence and seasonal activity of Cytospora leucostoma and C. cincta in french prune trees in California. Plant Dis. Rep. 60:106-110.

_____., K. Uriu, and F. J. Schick. 1976. Late season water deficits and development of Cytospora canker in French prune. Phytopath. Z. 72:305-314.

Boothby, D. 1983. Gummosis of stone-fruit trees and their fruits. J. Sci. Food Agric. 34:1-7.

Carroll, J. E., T. A. Tattar and P. M. Wargo. 1983. Relationship of root starch to decline of sugar maple. Plant Dis. 67:1347-1349.

Chalmers, D. J. and I. B. Wilson. 1978. Productivity in peach trees: tree growth and water stress in relation to fruit growth and assimilate demand. Ann. Bot. 42:285-294.

_____. and B. van den Ende. 1975. Productivity of peach trees: factors affecting dry-weight distribution during tree growth. Ann. Bot. 39:423-432.

Gairola, C. and D. Powell. 1971. Extracellular enzymes and pathogenesis by peach Cytosporas. Phytopathology 66:1318-1320.

Green, D. J., W. C. Shortle, and A. L. Shigo. 1981. Compartmentalization of discolored and decayed wood in red maple branch stubs. For. Sci. 27:519-522.

Grosclaude, C. 1966. La gommose des arbres fruitiers. Ann. Epiphytes 17:129-137.

Hampson, M. C. and W. A. Sinclair. 1973. Xylem dysfunction in peach caused by Cytospora leucostoma. Phytopathology 63:676-681.

Horsfall, J. G. and E. B. Cowling, eds. 1980. Plant Disease: an advanced treatise. Vol. V. How plants defend themselves. Academic Press, N.Y. 534 pp.

Houston, D. R. 1967. The dieback and decline of northeastern hardwoods. Trees 28:12-14.

Kuc, J. and L. Shain. 1977. Antifungal compounds. Vol. II. Interactions in biological and ecological systems, M. R. Siegel and H. D. Sisler, eds. pp.497-535.

Maggs, D. H. 1963. The reduction in growth of apple trees brought about by fruiting. J. Hort. Sci. 38:119-128.

McLaughlin, S. B. and D. S. Shriner. 1980. Allocation of resources to defense and repair. In: Plant Pathology: An Advanced Treatise (Vol. V). eds. J. G. Horsfall and E. B. Cowling. Academic Press, N.Y. pp.407-431.

Mitra, R. and C. R. Bhatia. 1982. Bioenergetic considerations in breeding for insect and pathogen resistance in plants. Euphytica 31:429-437.

Mochizuki, T. 1962. Studies on the elucidation of factors affecting the decline in tree vigor in apples as induced by fruit load. Bull. Fac. Agric. Hirosaki Univ. 8:40.

Okie, W. R. and C. C. Reilly. 1983. Reaction of peach and nectarine cultivars an selections to infection by Botryosphaeria dothidea. J. Amer. Soc. Hort. Sci. 108:176-179.

Priestley, C. A. 1970. Carbohydrate storage and utilization. In: Physiology of Tree Crops. eds. L. C. Luckwill and C. V. Cutting. Academic Press, N.Y. pp.113-127.

_____. 1965. A new method for the estimation of the resources of apple trees. J. Sci. Food Agric. 16:717-721.

Proebsting, E. L., Jr. 1958. A quantitative evaluation of the effects of fruiting on growth of Elberta peach trees. Proc. Amer. Soc. Hort. Sci. 71:103-109.

Rogers, W. S. and G. A. Booth. 1964. Relationship of crop and shoot growth in apples. J. Hort. Sci. 39:61-65.

Rosenberger, D. A. 1982. Biology and control of Cytospora Fang. in peach planting. N.Y. Food and Life Sci. Bull. 92.

Scorza, R. 1982. Resistance to Cytospora in stone fruit trees. Proc. Stone Fruit Decline Workshop. Oct. 18-20. Mich. State Univ., East Lansing.

Shain, L. 1979. Dynamic responses of differential sapwood to injury and infection. Phytopathology 69:1142-1147.

_____. 1967. Resistance of sapwood in stems of loblolly pine to infection by Fomes annosus. Phytopathology 57:1493-1498.

Shigo, A. L. and C. L. Wilson. 1982. Wounds in peach trees. Plant Dis. 66:895-897.

Shortle, W. C. 1979a. Compartmentalization of decay in red maple and hybrid poplar trees. Phytopathology 69:410-413.

_____. 1979b. Mechanisms of compartmentalization of decay in living trees. Phytopathology 69:1147-1151.

Stassen, P. J. C., O. Bergh, C. W. J. Bester and M. DuPreez. 1982. Reserves in full-bearing peach trees: carbohydrate reserves and their implications to orchard practices. Deciduous Fruit Grower Oct. 424-430.

Wareing, P. F. and J. Patrick. 1975. Source-sink relations and the partition of assimilates in the plant. In: Photosynthesis and Productivity in Different Environments. ed. H. P. Cooper. Cambridge University Press, N.Y. pp.481-499.

Wensley, R. N. 1966. Rate of healing and its relation to canker of peach. Can. J. Plant Sci. 46:257-264.

_____. 1970. Innate resistance of peach to perennial canker. Can. J. Plant Sci. 50:339-343.

Wilson, C. L., S. S. Miller, B. E. Otto and B. J. Eldridge. 1984. Pruning technique affects dieback and Cytospora infection in peach trees. HortScience 19:251-253.

_____., A. L. Shigo and P. L. Pusey. 1982. Long live the peach tree. Amer. Fruit Grower. Feb:22-24.

Wisniewski, M., A. L. Bogle and C. L. Wilson. (In Press). Histopathology of canker development on peach trees following inoculation with Cytospora leucostoma. Can. J. Bot.

_____., A. L. Bogle, W. C. Shortle and C. L. Wilson. 1984. Interaction between Cytospora leucostoma and Host-phenolic compounds in dormant peach trees. J. Amer. Soc. Hort. Sci. 109:563-566.

_____. 1983. Anatomical and physiological aspects of a host-pathogen interaction: Cytospora canker on Prunus persica (L.) Batsch. Ph.D. Thesis. Univ. New Hampshire, Durham.

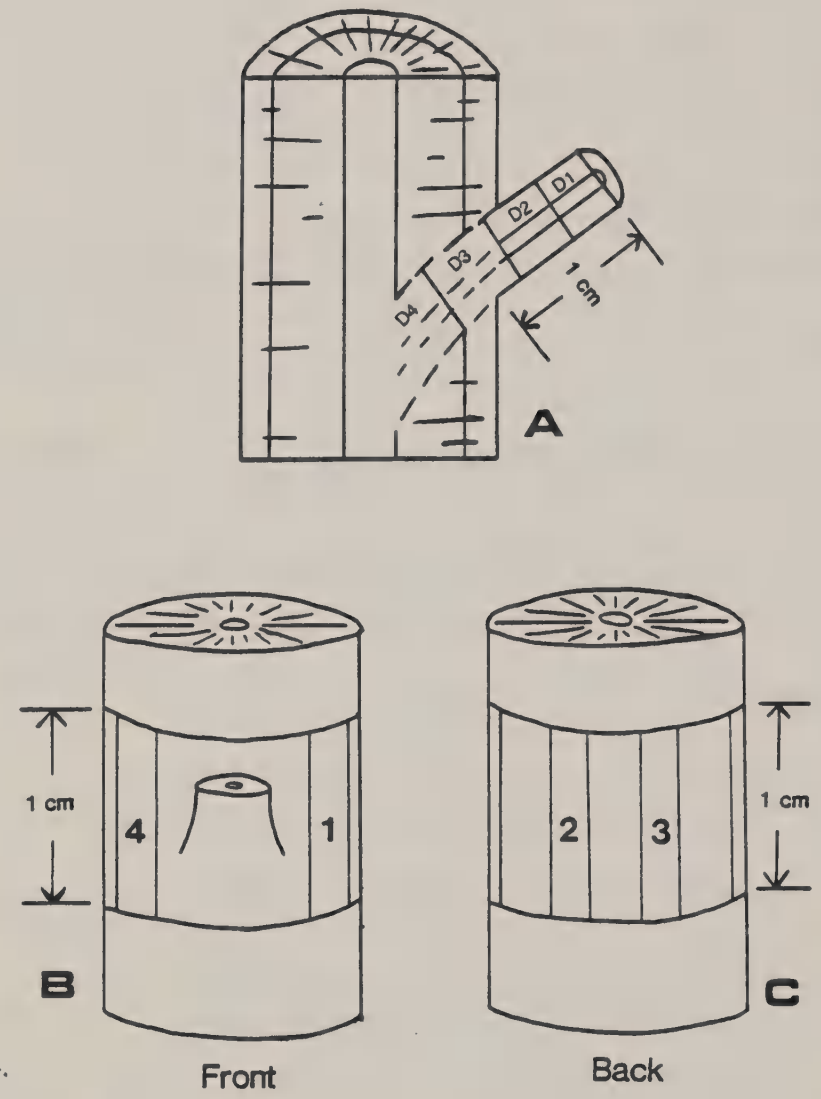


Fig. 1. Diagrammatic representation of sampling areas on short stubs (A) and main stems (B and C). Zones 1 and 4 on the main stem are referred to as contiguous to the inoculated short stub while zones 2 and 3 are referred to as being opposite.

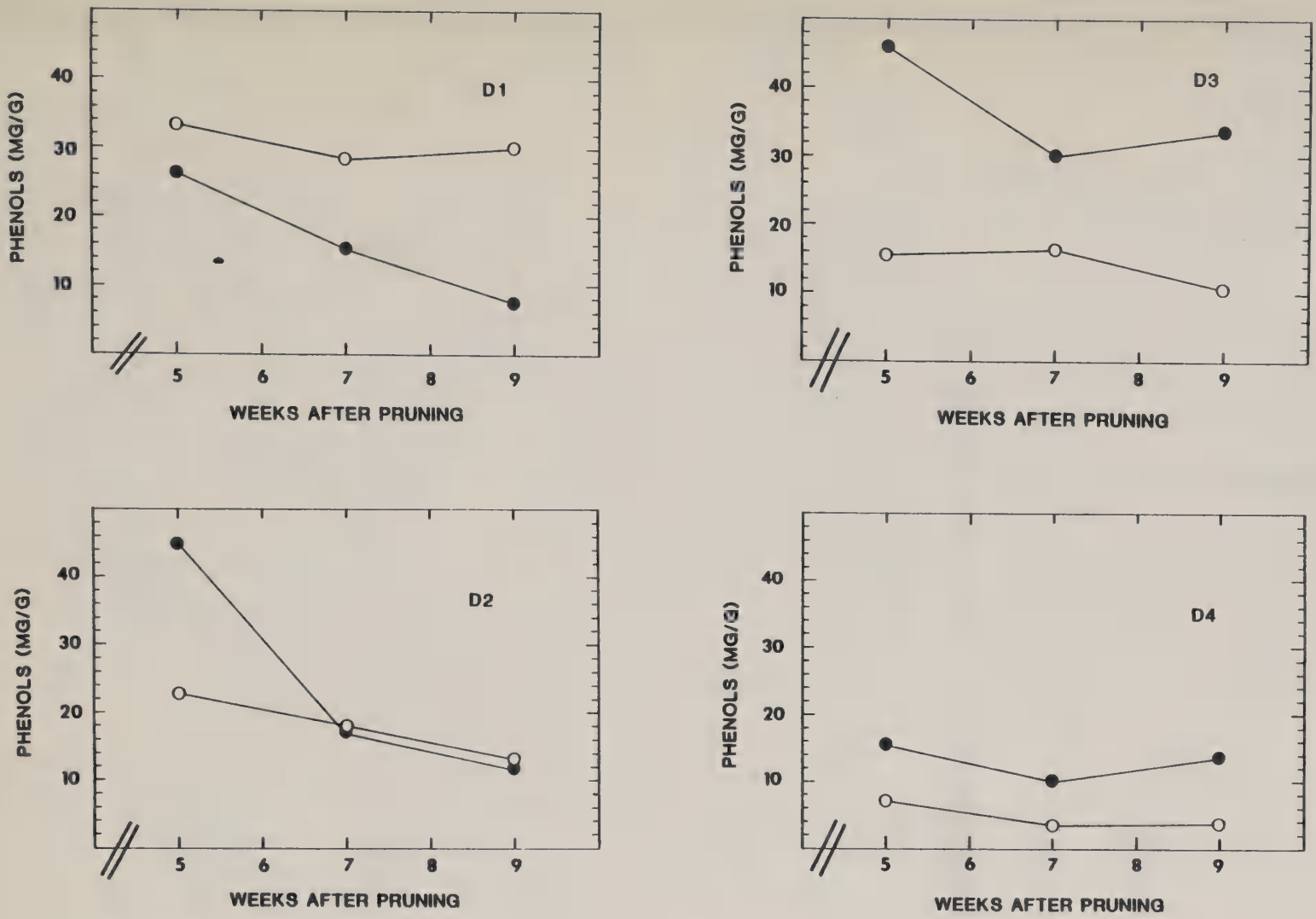


Fig. 2. Total extractable phenolics in wood tissue sections (D1-D4) of inoculated (●-●) and noninoculated (o-o) short stubs in dormant peach trees. n=6, for each sampling period. Values represent the mean ± SE for both cultivars combined. No significant differences existed between cultivars.

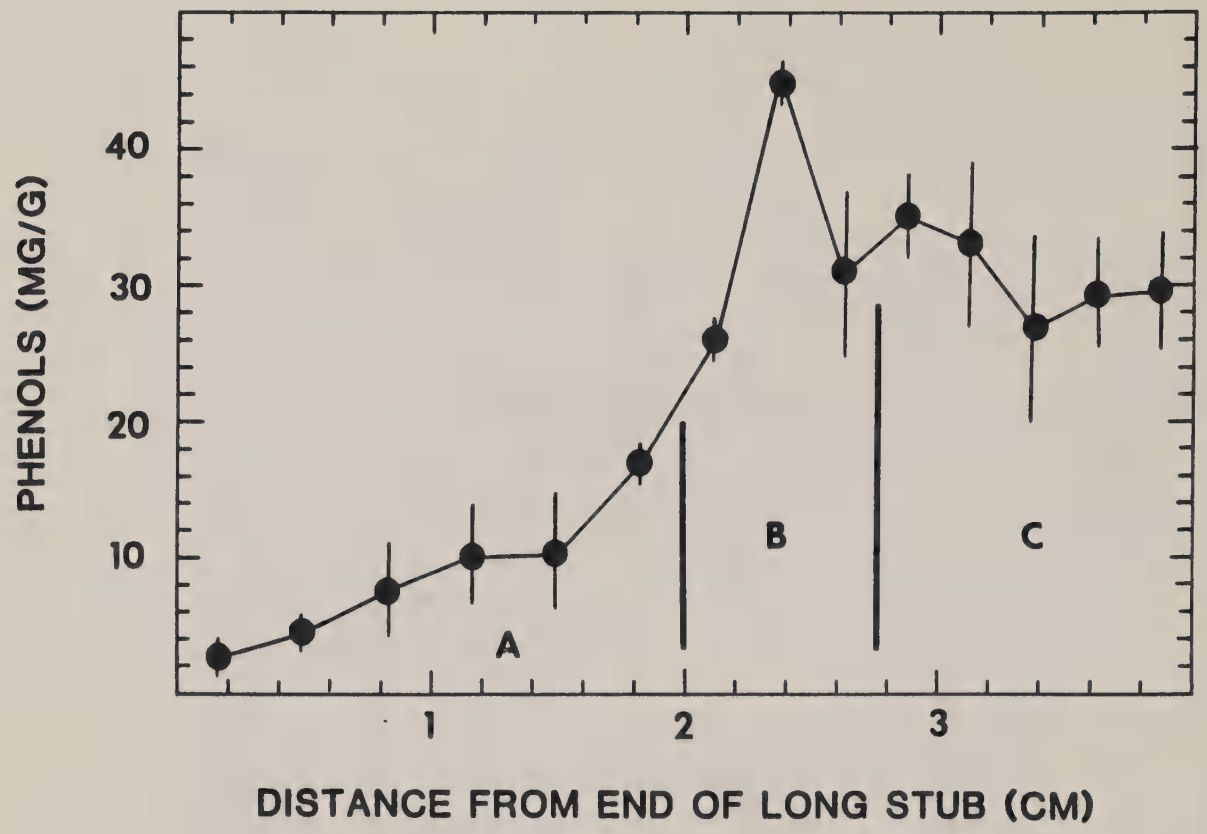


Fig. 3. Total extractable phenolics in combined wood and bark tissues of inoculated long stubs (cv Sunhigh) four weeks after inoculation. Labeled areas (A-C) correspond to morphological zones present in the long stub. A=necrotic zone; B=transition zone; C=living tissue zone. Data represents mean $\pm$ 1 SE (n=2).

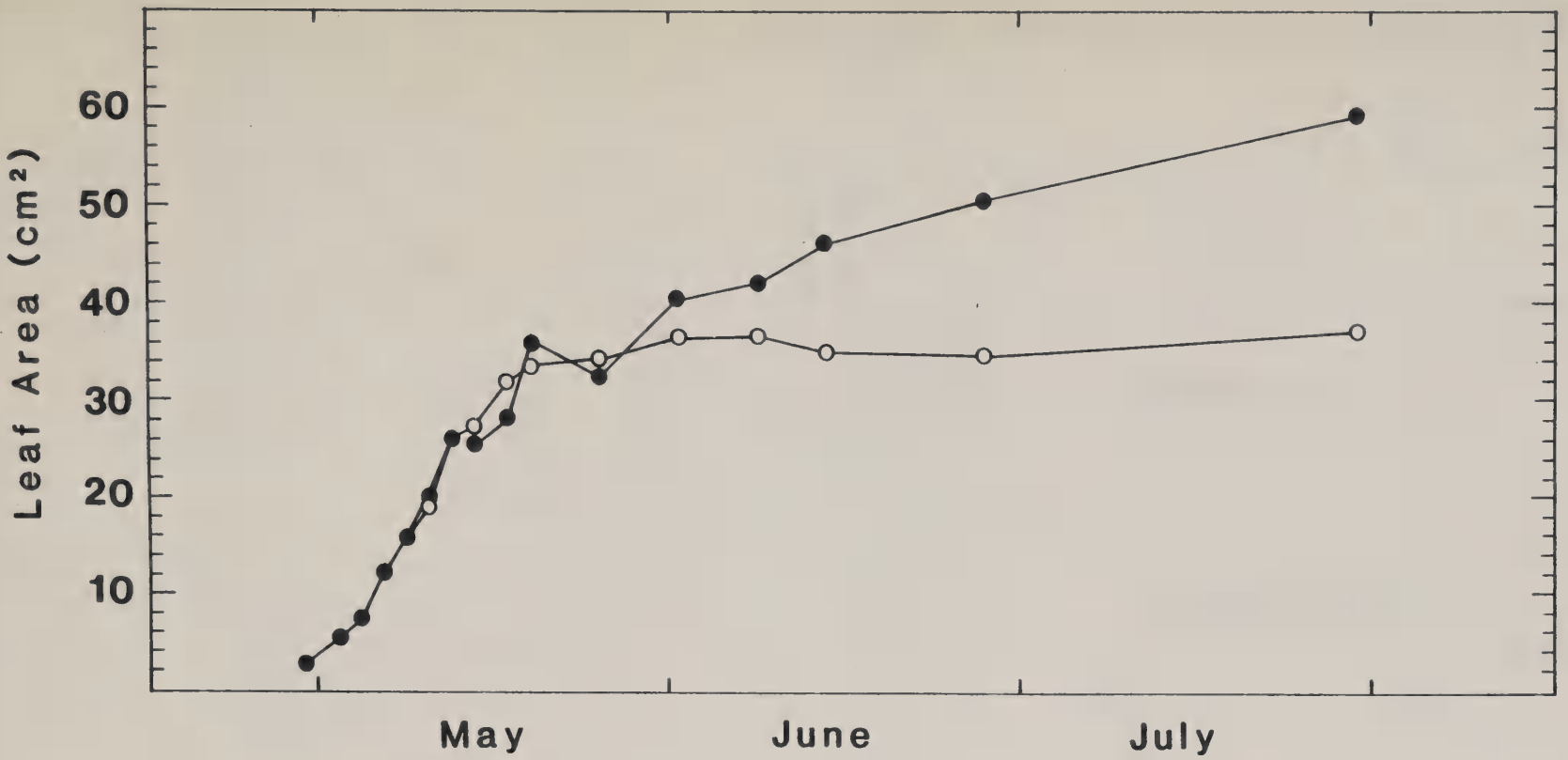


Fig. 4. Mean leaf area for fruiting (o-o) and deblossomed trees (●-●). Significant differences exist between the two treatments regarding overall patterns (2-way ANOVA, $p < .001$) and final mature leaf size (ANOVA, $p < .001$). Sample size was n=50 for each treatment at each time period.

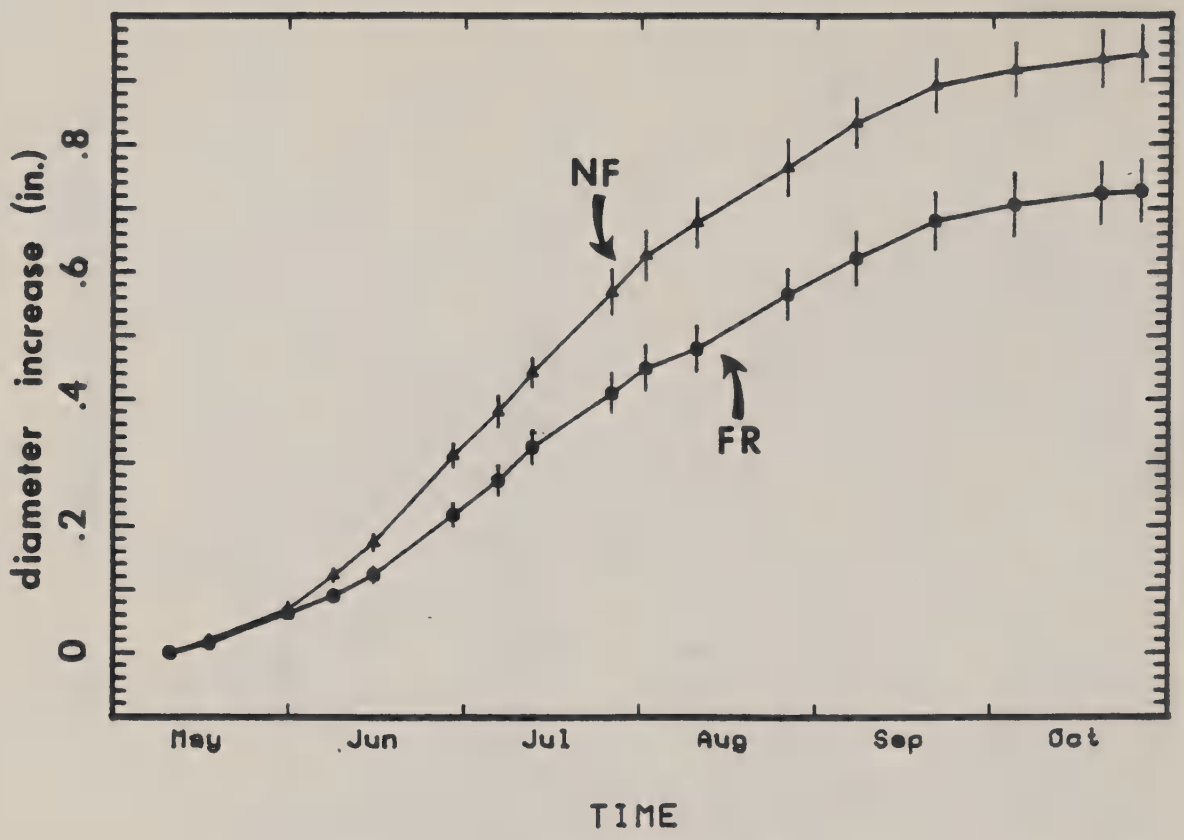


Fig. 5. Mean diameter increase (± se) in fruiting (FR) and deblossomed (NF) trees. Significant differences exist between the two treatments regarding overall patterns (2-way ANOVA, $p < .01$) and total diameter increase (ANOVA, $p < .01$). Sample size was n=5 and n=4, respectively.

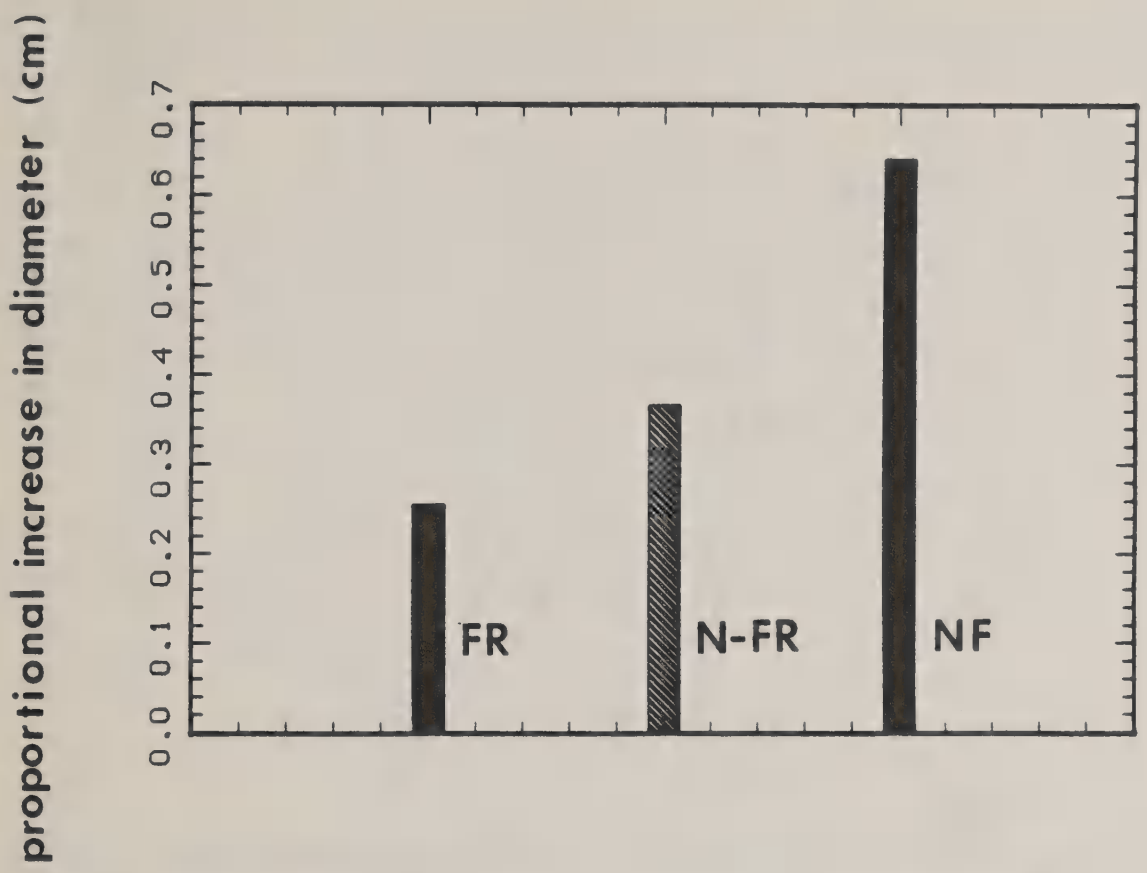


Fig. 6. Mean proportional increase in diameter for one-year-old twigs on deblossomed trees (NF), non-bearing twigs on fruiting trees (N-FR), and bearing twigs on fruiting trees (FR). Significant differences existed in total proportional increase between NF and FR (ANOVA, $p < .01$). Differences between FR and N-FR were not significant (ANOVA, $p < .05$). Sample sizes were n=24, n=20, n=23, respectively.

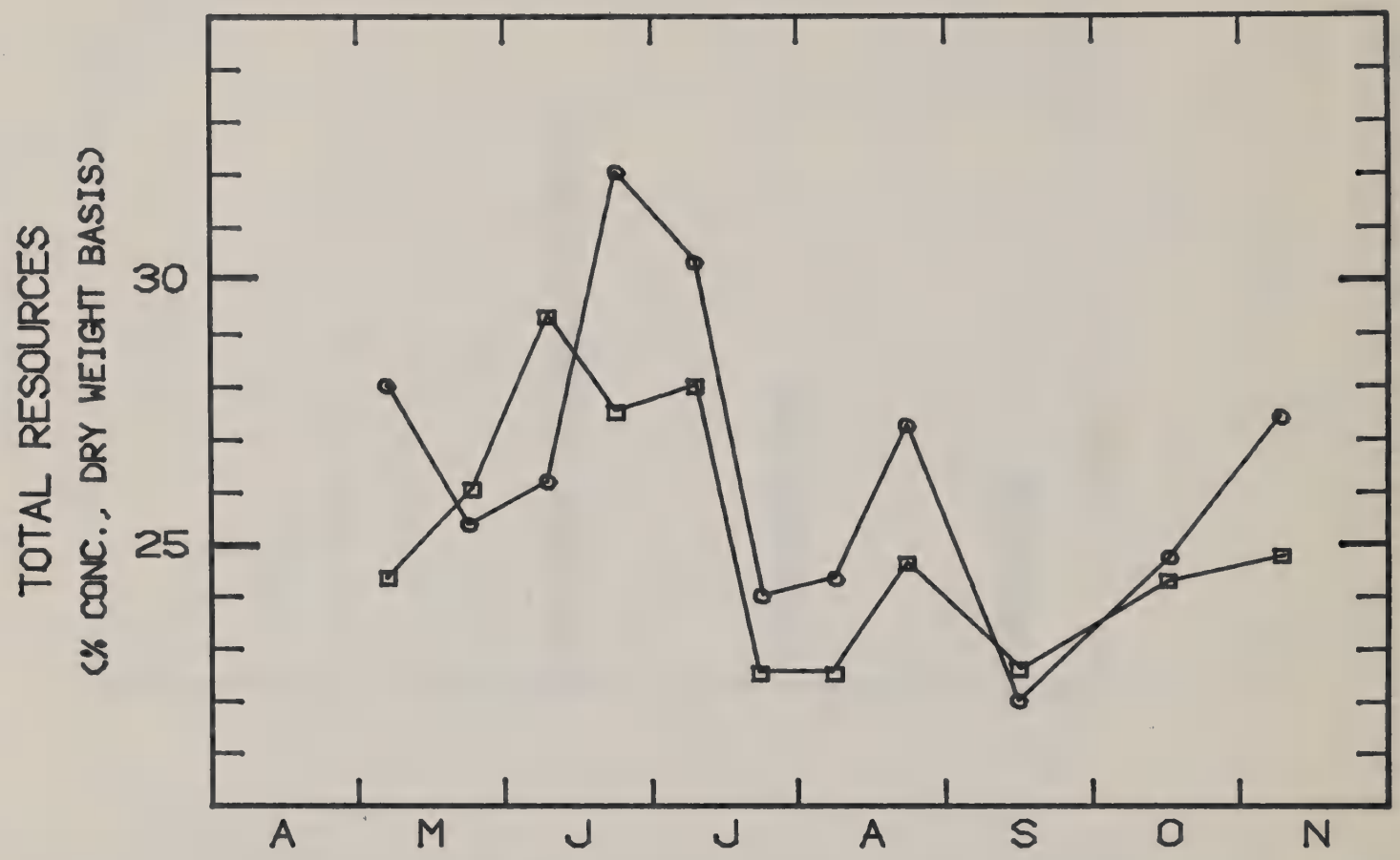


Fig. 7. Mean estimate of total resources in one-year-old twigs of fruiting (▫-▫) and deblossomed (o-o) trees. Significant differences existed between the two treatments regarding overall patterns (2-way ANOVA, $p < .025$) and final levels (ANOVA, $p < .05$). Sample size was n=10 for each treatment at each time period. Total resources are defined as all non-structural carbohydrates plus extractable hemicellulose (Priestley, 1965).

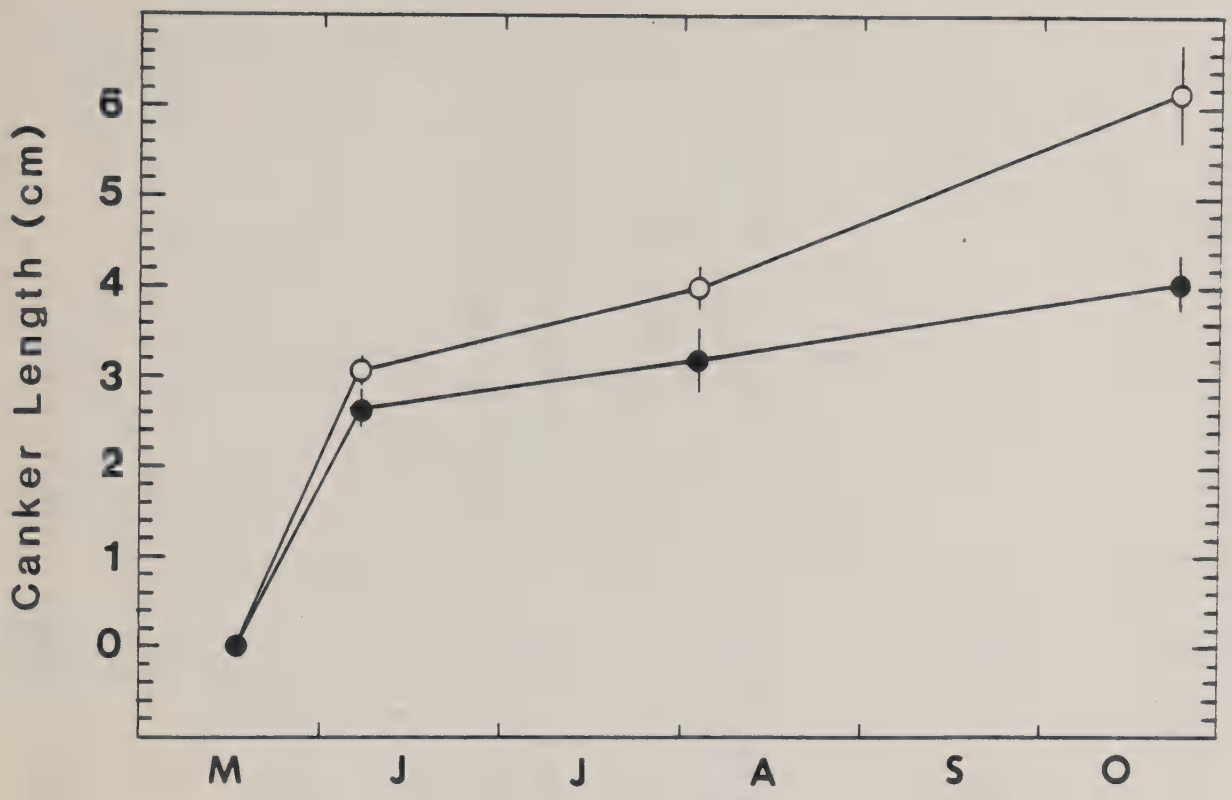


Fig. 8. Mean canker length (± 1 se) in fruiting (o-o) and deblossomed (●-●) trees. Significant differences existed between the two treatments regarding overall patterns (2-way ANOVA, $p < .025$) and final mean canker size (ANOVA, $p < .005$). Sample sizes were n=23, 16, and 15 for fruiting trees and n=22, 16, and 16 for deblossomed trees for the three sampling dates, respectively.

Table 1. Total extractable phenols in wood and bark tissues of nodal sections of the main stem at the time of pruning and nine weeks after pruning.

Zone[1]	Sunhigh			Loring		
	At Time of Pruning[2]	Nine Weeks[3]		At Time of Pruning[2]	Nine Weeks[3]	
	Control	Control	Inoculated	Control	Control	Inoculated
	mg phenol / g wood tissue					
1,4 Contiguous	11	13	31 7	9	25	84
2,3 Opposite	9	14	14 1	7	18	15
	mg phenol / g bark tissue					
1,4 Contiguous	73	98	175 24	64	114	206
2,3 Opposite	83	109	95 6	57	104	123

[1] No significant differences existed between zones 1 and 4 or between zones 2 and 3. Therefore, the means of zones 1, 4 and 2, 3 were pooled and treated as contiguous and opposite, respectively.

[2] Mean of 6 observations SE representing one sample from each of 3 trees. No significant differences existed between cultivars or zones (2-way ANOVA, p .25).

[3] Mean of 6 observations SE representing one sample from each of 3 trees. Significant differences exist in contiguous zones (1, 4) between inoculated and uninoculated samples for both wood and bark tissues. In addition, a significant difference exists between cultivars for wood tissues in contiguous zones (3-way ANOVA, p .05).

Table 2. Scoring Treatments of Cherry Trees in Row AAA.

Treatment	Scoring
1	Check
2	3 vertical scores, Spring 1984
3	3 vertical scores, Fall 1983
4	6 vertical scores, Spring 1984
5	6 vertical scores, Fall 1983

Mechanical Harvesting Treatment

	Row AAA	
	1983	1984
Harvester	USDA	Friday
Clamp	C	Tri
Pads	Kilby	Friday
Peak psi	250-350	1200
Shake, sec.	3	10
Time		a.m.

IS WOUND RESPONSE IN PEACH BARK RELATED TO RESISTANCE TO CYTOSPORA?

Alan R. Biggs, Research Scientist
Agriculture Canada, Research Station
Vineland Station, Ontario, Canada L0R 2E0

Introduction

The fungi which cause peach canker, Leucostoma cincta and L. persoonii, are considered to be weak pathogens because they require wounds to initiate infection. The importance of wounds in the peach canker pathosystem was originally realized by Willison (9). Weaver's studies on defoliation were the first to provide evidence that peach varieties may possess different levels of wound response capacity (6). More recently, the work of Shigo and Marx (4), Shigo and Wilson (5), Wilson (10) and Wilson et al. (11), has served to rekindle interest in wound response, particularly with regard to peach. A thorough understanding of wound response could provide insight into processes of pathogenesis and disease resistance, especially in weak parasite/wound interactions (3).

It has been suggested that varietal selection toward efficient xylem compartmentalization would result in increased disease resistance in peach (10). Although xylem responses are significant, bark tissue responses must be considered, also. Bark responses, generally described by some researchers as "wound closure" or "callusing", are actually quite complex and should be included with studies on compartmentalization in an organismal approach to disease resistance.

The complexities of bark response - what, when, and how to measure

When bark is wounded, in the absence of a pathogen, to a depth where only the phellogen is disrupted, a sequence of tissue reactions are observed that ultimately result in generation of a new phellogen beneath and around the wound. Response to deeper wounds where other generative or functional tissues are injured is more complex and won't be considered here.

In earlier studies on wound response in peach bark (1), I was able to define a sequence of anatomical and histochemical changes which characterize the events up to and including generation of new phellogen. After wounding, the following tissue changes are observed:

1. 0-3 days, desiccation of the first few layers of cells immediately internal to the wound surface; cell walls appear brown and test positive for increased phenols, total carbohydrate, and callose;

2. 3-7 days, the cell walls of a layer of cells, 3 to 4 cells thick, approximately 600-700 um internal to the wound surface become, at first,

impregnated with wall-bound phenolic acids which, soon after, polymerize to form guaiacyl-unit lignin (phloroglucinol test positive);

3. 7-10 days, a thin (0.3-0.5 um) lining of suberin forms on the inner surface of the cell walls of the innermost lignified cells;

4. 4-10 days, cell dedifferentiation takes place immediately internal to the lignified area but the final redifferentiation to form new phellogen is observed only after the deposition of suberin linings in the boundary zone;

5. 10-14 days, the new phellogen generates suberized necrophylactic phellem; and

6. 17 days and on, the area external to necrophylactic phellem continues to desiccate, becomes crushed by outwardly expanding tissues, and is eventually sloughed.

The initial formation of the impervious boundary varies in time and space. It forms first in phloem ray parenchyma beneath the wound; it forms last in the phelloderm region of the original injured periderm. In my assessments of bark response, I've chosen the latter area to measure my variables because it is where continuity between old and new tissues is re-established and it is a particularly susceptible tissue to fungal colonization. Continuity is defined as complete suberin connection between old and new tissues.

Re-establishment of suberin continuity should be the point when a shallow bark wound is considered closed. To measure when this has taken place, a new method for detecting suberin had to be developed. By reacting phloroglucinol + HCl or toluidine blue O with the lignin of the boundary zone, followed by observation of tissues under ultraviolet epi-illumination, autofluorescence of lignin was quenched which allowed for clear, unimpeded observation of suberin linings in the boundary zone (2). A microscope photometer was employed to measure autofluorescence intensity of glycerol mounts (non-quenched) and toluidine blue O mounts (quenched) of peach bark taken at intervals after wounding. The results are presented as suberin and total boundary zone autofluorescence.

Procedure - wounding and sampling

Three replicate trees of each of seven varieties thought to represent a range of susceptibility to the peach canker fungi were selected for wounding only. The field grown trees were of variable age so an effort was made to only wound tissues thought to be of similar age (ca. 5 years old).

A 4 mm diameter cork borer was used to injure the phellogen and cortex to a depth of about 2 mm. Five wounds on one scaffold limb per tree were made 10 cm apart and were placed in a semi-spiral pattern so that one wound was not directly above or below another wound. After 3, 7, 10, 14, and 17 days, the wounds were removed with a larger diameter cork borer to the depth of the cambium. Each bark disk was halved longitudinally, placed in FAA, dehydrated in t-butyl alcohol, embedded in paraffin, and sectioned with a rotary microtome at 8 um thickness. Ribbons were mounted on glass slides, deparaffinized in xylene, and mounted in glycerol or toluidine blue O. Photometer measurements were taken to quantitate total boundary zone and suberin autofluorescence, respectively. Non-suberin autofluorescence was obtained by subtraction.

The wound series on the seven varieties was performed three times (June 12, July 10, and August 1, 1984). Each wound series was performed on a different scaffold limb on the same three trees of each variety. Each photometer observation repesents an average of five measurements taken on serial sections from one slide. Final autofluorescence intensity was calculated by subtracting the fluorescence intensity of non-wounded control tissue from the fluorescence intensity of the wounded tissue. All scaffold limbs in the experiment were free of Cytospora cankers. Cankers distal to the wound sites were presumed not to have a significant influence on wound response because, during the growing season, they are effectively compartmentalized. Total number of cankers distal to the wounds were counted at the end of the experiment and total canker numbers were used to rank the seven varieties. The seven varieties were also ranked at each wound age according to the pooled data for suberin and non-suberin autofluorescence intensity and Spearman's rho was used to assess the correlation between autofluorescence intensity and number of cankers.

Results and discussion - what next?

Figures 1 and 2 represent the pooled data for non-suberin and suberin autofluorescence intensity at each of five wound ages. Analysis of variance on the suberin data followed by mean separations for the different varieties show that V68101 had the most rapid wound response and HW233 had the least rapid wound response. The other varieties fall in between these extremes in the following order: V68101, V75101, Boone Co. Seedling source 1, Boone Co. Seedling source 2, Ellerbe, Candor, and HW233.

When total number of cankers distal to the wound treatments for each variety are ranked and compared to the varietal rankings of non-suberin and suberin autofluorescence intensity (Table 1), we find that the correlation between suberin autofluorescence intensity and canker number is highly significant (P=0.01) when measured at 7, 10, 14, or 17 days after wounding. None of the non-suberin autofluorescence intensity data show a highly significant correlation with the canker rating.

These results provide evidence to suggest that rate of impervious tissue formation and phellogen generation at wounds could be useful indicators of cultivar susceptibility to the peach canker fungi. These findings are in agreement with those of Wensely (7) who reported that wound closure was related to field performance. In a later study, Wensley (8) reported that "innate resistance" was unrelated to field performance and unsuitable as an index of performance in the orchard. His results were based on percent infection and canker growth using four cultivars with a range of susceptibility based on field performance. However, bark histochemical parameters were not assessed and non-inoculated control wounds were not included in the study.

The present study supports the findings of Weaver (6) and Wensley (7) and demonstrates that wound response assessments can be made over a shorter time period than that used in previous studies where wound closure was assessed. Re-establishment of suberin continuity and suberin autofluorescence intensity in impervious tissue and necrophylactic periderm could prove to be effective measures of cultivar susceptibility to peach canker fungi.

Peach canker research at Vineland will continue to delve into the relationship between wound response and susceptibility to disease. An understanding of phellogen generation is the first step in this process. Future investigations will address aspects of vascular cambium generation and functional sapwood response. For example, our method of suberin detection has allowed observation of xylem ray suberization in response to wounds in walls 1, 2 and 3 of the CODIT model (4). Xylem ray suberization occurs in the region of xylem vessel blockage and preliminary results show that it is also quantitatively associated with field performance. There are several questions which must be addressed including: 1. What degree of bark wound response is associated with disease resistance? 2. Are bark and xylem responses in peach genetically independent? 3. What enzymic changes occur during boundary zone formation? 4. Could enzyme assessments provide a more rapid means of assessing wound response capacity? 5. What environmental variables influence wound response capacity and to what degree? 6. Can wound response capacity be modeled mathematically in order to predict periods of susceptibility?

The answers to these questions may someday help to complete our understanding of wound response and pathogenesis in trees and could provide a valuable framework to initiate a program for breeding for disease resistance in peach to the canker fungi.

Literature Cited

1. Biggs, A.R. 1984. Boundary zone formation in peach bark in response to wounds and Cytospora leucostoma infection. Can. J. Bot. 62:2814-2821.

2. Biggs, A.R. 1984. Intracellular suberin: Occurrence and dectection in tree bark. I.A.W.A. Bull. n.s. 5:243-248.

3. Mullick, D.B. 1977. The nonspecific nature of defense in bark and wood during wounding, insect, and pathogen attack. Recent. Adv. Phytochem. 11:395-441.

4. Shigo, A.L. and H.G. Marx. 1977. Compartmentalization of decay in trees (CODIT). U.S. Dept. of Agric. Bull. 405. 73 pp.

5. Shigo, A.L. and C.L. Wilson. 1982. Wounds in peach trees. Plant Disease 66:895-897.

6. Weaver, G.M. 1963. A relationship between the rate of leaf abscission and perennial canker in peach varieties. Can. J. Plant Sci. 43:365-369.

7. Wensley, R.N. 1966. Rate of healing and its relation to canker of peach. Can. J. Plant Sci. 46:257-264.

8. Wensley, R.N. 1970. Innate resistance of peach to perennial canker. Can. J. Plant Sci. 50:339-343.

9. Willison, R.S. 1963. Peach canker investigations. II. Infection studies. Can. J. Res. 14:27-44.

10. Wilson, C.L. 1982. Peach tree wounds and decline. Proc. Stone Fruit Decline Workshop (Oct. 18-20), Mich. State Univ. East Lansing, MI.

11. Wilson, C.L., S.S. Miller, B.E. Otto, and B.J. Eldridge. 1984. Pruning technique affects dieback and Cytospora infection in peach trees. Hortscience 19:251-253.

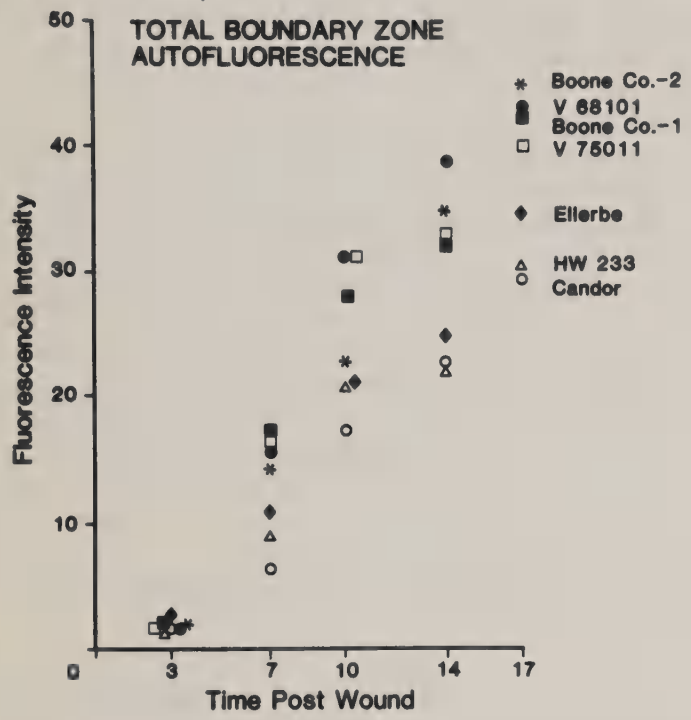


Figure 1. Total boundary zone autofluorescence intensity of pooled data for seven peach varieties wounded in June, July and August 1984.

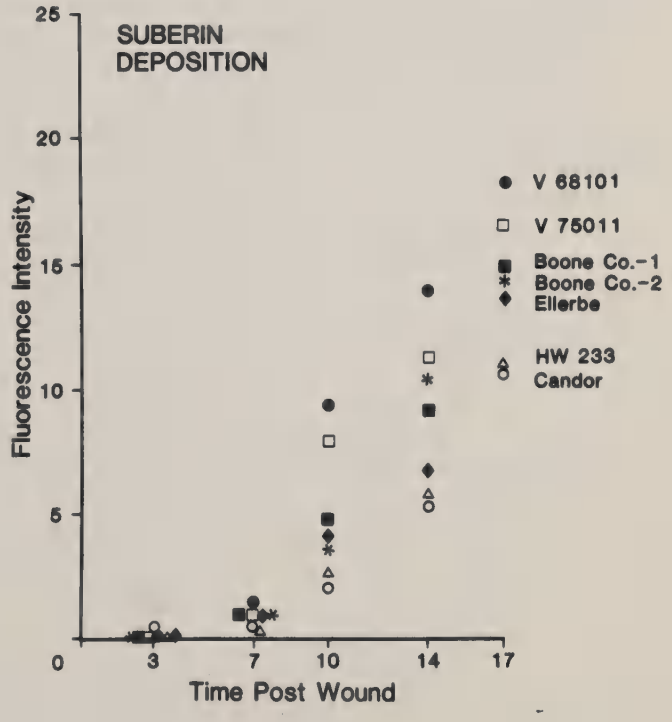


Figure 2. Suberin autofluorescence intensity of pooled data for seven peach varieties wounded in June, July and August 1984.

Table 1. Spearman's correlation coefficient for pooled suberin and non-suberin autofluorescence intensity versus number of cankers on peach scaffold limbs distal to wound treatments.[a]

Wound age (days)	Spearman's r	
	Non-suberin autofluorescence intensity	Suberin autofluorescence intensity
3	- 0.803[b]	- 0.807
7	- 0.793	- 0.921**
10	- 0.878*	- 0.950**
14	- 0.835	- 0.907*
17	- 0.779	- 0.921**

[a] Ranks for non-suberin and suberin autofluorescence intensity by wound age for seven peach clones versus ranks for total number of cankers on all wood distal to the wound treatment area.

[b] Spearman's r; *, ** = significant correlation at p = 0.05 and 0.01.

HOW TREES SURVIVE AFTER INJURY AND INFECTION

Alex L. Shigo
Northeastern Forest Experiment Station
USDA Forest Service, Durham, NH 03824

Abstract.--Trees survive after injury and infection so long as they have the time, energy reserves, and genetic capacity to compartmentalize injured and infected tissues rapidly and effectively to small volumes, and to generate enough new tissues in new spatial positions to store enough energy to maintain the tree. Trees have many protection features and a defense system. The defense system is centered about compartmentalization, which is a boundary-setting process to resist spread of pathogens. Tree pathogens survive so long as they can spread fast and far enough to gain enough space and energy to reproduce. Trees and pathogens interact under the constant pressure of the ever-changing environment. A tree is reexamined from the view of its boundary-setting defense system. When branch development is clarified, proper pruning methods become obvious. When boundary-setting is understood to be under strong genetic control, trees resistant to spread of decay can be selected for our orchards. When tree decay is clarified, the major cause of damage to trees worldwide can be reduced.

Additional key words: Compartmentalization, discoloration and decay, tree defense.

SOME PROBLEMS AND SOLUTIONS

Tree decay is a major worldwide cause of injury to trees in forests, parks, cities, and orchards. The major problem is that decay is considered a natural process beyond our regulation. Orchard managers accept it and try to work around it.

Some textbooks still in use (Boyce 1961) state that decay is not a disease because it affects only the dead, nonresponsive heartwood. Most orchard managers learned about decay by being taught the heartrot concept. The heartrot concept is an excellent beginning concept, but it does not include the response of the living tree to injury and infection. It is a concept dealing primarily with types of wood decomposition, and the fungi that cause decay. Decay is considered a problem mainly on old, over-mature trees.

Improper pruning is a major worldwide problem. Pruning is one of the most common tree treatments, and the oldest agricultural treatment. For over 200 years, the recommendation has been to cut living and dead branches as close as possible to the joining stem--a flush cut--and then coat the wound with some wound dressing.

The flush cut branch is the most injurious treatment man has ever inflicted on trees worldwide. At least 14 serious tree problems, including rapid development of decay and cracks, can be started by flush cuts. Flush cuts were thought to stimulate the wounded trunk to form large callus ribs that were considered a sign of rapid healing. Because it was well documented over a century ago by Mayer-Wegelin (1936) that trees that had flush cut branches had large pockets of decay, a wound dressing was applied. The thought was that the flush cut solved the branch-healing problem--large callus ribs--but some materials were needed to solve the internal decay problem. Today flush cuts are still the rule, and the search for the perfect wound dressing that will stop decay continues. No data show that any material prevents or stops decay (Shigo and Shortle 1983).

The real problem behind the improper pruning techniques is that branch development has not been described clearly. Diagrams of branches in books before 1900 and still today show the trunk xylem tissues above a branch connecting with the xylem in the branch. Branch and trunk xylem tissues meet only below the branch. The natural shedding line at the branch base was known a century ago (Mayer-Wegelin 1936), but more emphasis then was on healing than on natural shedding. Once branch development and natural shedding are clarified, proper pruning becomes obvious. Pruning cuts should never injure or remove the branch collar that is at the branch base (Shigo 1983).

The problem with wound dressings and with many other tree treatments center about the desire to treat trees like people. People who work with trees have borrowed too much from the people who work with animals. Indeed, trees are different. The wound dressingss and healing rationale fit for animals, but not for trees.

Another problem is that trees are big organisms and their size makes it very difficult for intensive internal studies. It was not until the powerful chainsaws were made after 1940 that it was possible to systematically dissect large trees. Until then our concepts of trees and defects were primarily from the end of logs.

The major advancement with all of humankind came about when the benefits of health were realized. The same must be done now with trees. It is better to focus on what keeps you healthy, than on what makes you sick. We need to know more about what keeps trees healthy and concentrate on this, rather than continue to fight the many very obvious secondary agents that always follow poor health. We must examine the tree and its suvival systems.

A very old problem centers about the belief that wood is dead, nonreactive material. Most of the decay story was about heartwood. Heartwood was and still is considered a dead, nonreactive tissue. It may be dead according to our definitions, but it is surely a tissue that will react to injury and infection. Heartwood in most trees has a built-in protection feature--extractives--but it also has a system that reacts to resist spread of pathogens. Discolored wood has often been confused with heartwood, and the types of discolored wood have been given names that imply a type of heartwood--wound heartwood, false heartwood, pathological heartwood.

Too often color has been the major factor used to determine a type of wood. All wood darker than the sapwood was called heartwood or a type of heartwood. The term discolored wood is an extremely poor term (and I feel badly that I have helped to set it so firmly in the literature). A better general term would be "injury-altered wood." A long gradation of changes starts in the wood--sapwood, heartwood--after injury and infection. Some injury-altered wood is much less protective than contiguous wood. A great amount of confusion about wood could be clarified if wood anatomists and physiologists were presented with a much clearer explanation of wood changes that occur after injury and infection.

When a branch or root dies, there is an opening into the tree that can be infected by organisms. Dying roots and branches are the most common infection courts for tree-inhabiting organisms. Yet these courts are often not recognized as starting points for defects.

Because of the many microorganisms that infect trees, it has been assumed that it is impossible to select trees resistant to decay. In part this is correct. Trees cannot prevent infections in wood killed by wounds or in dying branches and roots. But, trees can resist spread of microorganisms. It is possible to select individuals within a species that set boundaries very rapidly and effectively to resist spread of microorganisms. This feature seems to be under moderate to strong genetic control.

Compartmentalization has been misunderstood as a process that always stops microorganisms, and succession has been misunderstood as a process where bacteria always infect first, then followed by non-hymenomycetous fungi, and finally the infection of Hymenomycetes. First, compartmentalization is definitely not an absolute process. It does not function perfectly all the time. When it does not function perfectly, some tree part, or the entire tree may die. Some microorganisms have developed effective ways to grow around boundaries. Second, succession is a highly ordered sequence of many microorganisms that assures survival of the groups. Hymenomycetes may be the first to interact with the tree--pioneers--or other types may be pioneers. The point is that many microorganisms are involved in the processes that may or may not result in total decomposition of wood.

Trees have many protection features and a defense system made up of many parts. Time is the name of the game. The tree and the tree-inhabiting organisms must stay alive long enough to reproduce. The time game is played against the environment. Trees must have superior protection features and a strong defense system to live and maintain reproduction.

I believe that the basic worldwide problem in the tree industries is the lack of understanding of what a tree is and how it functions to survive after injury and infection. The more tree managers know about trees, the better the decisions will be in favor of healthy trees.

The purpose of this paper is to reexamine a tree from the view of its defense system, which is compartmentalization (Shigo and Marx 1977, Shigo 1984). Compartmentalization is a boundary-setting process to resist spread of pathogens.

IN THE BEGINNING

Plants that were to become trees started to grow tall and massive on land over 400 million years ago. Our extensive coal deposits were formed by masses of fallen trees over 300 million years ago. Trees as we know them today began to develop 200 million years ago. Trees as we see them today have changed little over the last 50 million years.

Tree survival

To survive is to stay alive. After injury and infection, some individuals of a species die and some stay alive. The species exist only so long as some individuals stay alive long enough to reproduce. It is possible that some individuals of a species stay alive long enough to reproduce because they have not been seriously injured or infected. This is highly unlikely with trees. Unlike animals, trees cannot move away from a destructive agent. Animal survival strongly depends on the ability to move away from destructive agents. Trees have evolved under the constant pressures of all types of wounding agents--fire, storms, animals,

insects, volcanoes, man--and all types of organisms that could digest the tissues exposed by wounds. The forms and functions of trees are adaptations to the survival threatening pressures of injuries and infections, and to the ever-changing environment over very long periods. If this were not so, trees would not be here today. Not only are trees still on earth, but they continue to be the tallest, most massive, and longest lived organisms. To be so tall and massive for such a long time must require unique protection features--static state--and superior defense systems--dynamic state.

HYPOTHESIS

If trees do have unique protection features and superior systems for defense that makes long survival possible, and if trees' form and functions are successful adaptations for survival after many types of injuries and infections, then the best way to understand a tree is to study its response or reaction to the many types of naturally caused and experimentally caused injuries and infections.

Indeed, long-term environmental pressures also played a role in shaping the forms and functions of trees. But, if trees did not stay alive long enough to reproduce after injury and infection--relatively short time--the long term effects of the environment would not take place.

RESEARCH BACKGROUND

I have tried to test the hypothesis over a 25-year period. The research background or data base for this discussion comes from studies on the systematic dissections of thousands of trees that had naturally caused internal defects and changes in wood started by a great variety of experimentally inflicted wounds, in which many were inoculated by organisms. Also, systematic isolations of microorganisms were made to show temporal and spatial relationships after injury. Then these data and data from the studies of many other investigators were connected to expand the concept of tree decay. The living tree and its survival features were brought into the concept. The expanded concept included compartmentalization--the orderly response of the compartmented living tree to injury and infection--the microbial succession--the orderly sequence of many microorganisms in the process of decay. Then a model of compartmentalization of decay in trees--CODIT--was developed (Shigo and Marx 1977). New electrical tools and methods were developed with the help of many people (Shigo and Shortle, in press). Now the expanded concept, the CODIT model, and the electrical methods are being used to reexamine trees, many tree problems, and tree treatments.

In the beginning of our research program, emphasis was on the development of internal defects. As this sphere or circle of understanding increased, it was apparent that more had to be learned about the tree. As the basics of tree biology were studied, another sphere of understanding began to develop. Now the two spheres have combined, and they reinforce each other.

BASIC TREE BIOLOGY REVISITED

Trees are perennial, woody, compartmented, shedding plants that are usually, (but not always) tall, massive, long-lived, and single-stemmed. The first four characteristics are consistent and the most important.

The unique feature of a tree that separates it from all other organisms is its superior mechanical support. The combination of cellulose and lignin in the wood cell walls, and the cell arrangements, give trees their unique feature. In a teleological sense, the first priority of the evolving tree was to protect and defend its unique feature. If the evolving tree were not successful at this, then the tree plant would be similar to other nonwoody plants. This is an oversimplified statement to clarify some misconceptions about trees.

Decay-causing microorganisms are pathogens, even if they are attacking only the heartwood (whatever definition you have for heartwood). Pathogenesis is defined on the basis of the entire organism. If mechanical support is disrupted, the unique feature of a tree is disrupted, and the tree is back to a small nonwoody plant. Trees, like all organisms, die three ways: mechanical disruption (accidents, wounds, etc.), dysfunction, and infection (an agent blocks or takes energy). Any agent that causes the tree to fail and die is indeed a pathogen.

The tree has many built-in protection features--for example, bark with suberin, heartwood extractives, low nitrogen to high carbon content of wood, arrangements of cells, arrangements of microfibrils--but these protective features are only the first line for survival. A second line starts after injury and infection. The tree cannot restore--repair, replace--an injured xylem cell. But it can set boundaries to resist the spread of pathogens from injured to sound cells. If trees had only one line of protection features, it would not be long before the microorganisms would adapt and break them down. The evolving tree developed a dynamic defense system to resist or stall the microorganisms that were able to spread within the wood that had the protection features. The microorganisms survived by attacking in succession. If the tree did not have a second line of defense, the mechanical support system would be disrupted rapidly and the tree plant would be back to low plant again.

It is important to keep in mind that no matter how strong the protection features and the defense systems become, they cannot stop all organisms all the time. The same for organisms, no matter how aggressive they become as individuals or in succession, they cannot digest all tree tissues all the time. If this did happen, it would only happen for one period and the host and pathogens would no longer exist. Compartmentalization makes it possible for host and pathogens to survive. The pathogens do digest the tree, but digestion of tissues is regulated with generation of new tissues in new spatial positions. This interaction has the capacity for long-term survival.

Trees and pathogens have a common enemy and friend in the environment. In a sense, trees and pathogens "need" each other as a common orderly life force to survive against the short-termed disordered environment. The view of host, pathogen, and environment is different from the view that shows the three equally interacting with each other as a triangle. In the view I present, the host and pathogen represent the life force against the nonlife force of the environment. This is not to say that the environment does not affect the host and pathogen each in a positive or negative way, or both positive or both negative. A force is measured at the plane where two opposing pressures meet. If environmental pressures eliminated tree-inhabiting organisms, I doubt that trees would survive alone against the environment. The same can be said for tree pathogens. Time is the important factor. So long as the tree and pathogens have time to survive long enough to reproduce, the complex but orderly natural system will persist.

The built-in static protection features and the dynamic defense system are the keys to long-term survival for trees after injury and infection. As stated, if the protection features were the entire survival system, it would not be long before the microorganisms would adapt to it and digest it. Any feature that "stands still" is an easy target. The long-term survival advantage of the defense system is that it does not occur until after injury and infection. An organism cannot adapt to something that is not there at the time it arrives. The defense system of each tree will always be slightly different because of genetic programming. The organism that is able to survive the competition on the wound surface may not have the genetic capacity to interact with the internal defense system of a tree.

The wood-inhabiting microorganisms have a double problem. They must compete effectively with many other microorganisms on the fresh wound surface and then grow into the tree against the protection features and the defense system. Many of the microorganisms that can survive on the wound surface are poorly adapted to survival inside the tree. Once in the tree, the pathogens must interact with the tree and also defend itself and its occupied space from other microorganisms.

When the protection features, defense systems, competition, and the ever-changing environment outside and inside the tree are considered, it is a miracle that some microorganisms do survive inside the tree.

If the microorganisms can be resisted long enough for the tree to generate enough new cells in new spatial positions, then the tree wins that battle.

Some defense systems had to evolve that were ready to react after injury and infection to resist rapid spread of microorganisms. The defense system had to function where there were still some cells with living contents and aged cells without living contents.

Every tree has some microorganisms that will attack its injured or dying sapwood or injured heartwood, regardless of amounts of extractives. Extractives do stall the growth of microorganisms and some trees have an extremely effective extractive-based protection system. After injury and infection, moisture content near wounds and in dying cells begin to change. Changes also occur in pH and concentration of microelements. In some trees, wood is infected by bacteria and wetwood results. Wetwood seems to inhibit infection by decay-causing fungi, and the mechanical support system is protected. In summary to this point, many different types of protection features make wood difficult to digest by microorganisms, and boundary setting limits the spread of the digestion when it does occur. This double-powered approach buys time for the tree to continue generating new cells in new spatial positions. Yet, wood-inhabiting microorganisms do digest trees when time is extended.

In the section on problems, a point is made that heartwood is considered a dead, nonreactive tissue. And yet heartwood does discolor when injured and infected. If discolored wood is wounded, it does not discolor further. Discolored wood has reached the lowest limit of energy reaction; heartwood has not. Heartwood will not only discolor after wounding, but also it will discolor in orderly predictable patterns depending on the wound. Heartwood may not contain cells with living contents, but it may contain some materials that are not at the lowest energy state. Because heartwood was always considered a dead, nonreactive tissue, no research was done to investigate its ability to react to resist spread of microorganisms.

If the heartrot concept and heartwood were not able to resist spread of microorganisms by setting boundaries, disordered columns of decayed wood would be in heartwood. Yet the patterns of decayed wood in heartwood follow the CODIT patterns for trees that do not have a heartwood. I believe we have a situation with heartwood where our definition or concept of dead do not apply. It may be that the cells are chemically cocked, as a mousetrap, when they reach a point of genetically controlled aging. When the "trigger" is hit, the trap reacts in a predictable way, and usually kills the mouse. The mousetrap is not alive. But once cocked, it can react once. It takes energy to cock the trap. There may be some energy in "cocked" chemicals in sound heartwood.

Discolored wood is even more confusing than heartwood. There is no one type of discolored wood. Discolored wood is like a color spectrum or rainbow. It is the condition of a tissue in transition. It is a gradation of changes. Too much emphasis has been placed on color and color changes. Discolored wood is wood altered as a result of injury and infection. After a tree is wounded, or after a branch or support root begins to die, contents of living cells begin to change, usually to a protective state. The changes are the result of tree-microorganism interactions. Discolored wood is usually more protective soon after it is formed, and then it may or may not begin to become less protective as different microorganisms infect. It is very important to separate cells that die and discolor, from cells that discolor and die. The latter is more in tune with hypersensitive reactions when the organism kills portions of itself to save the whole. In other instances, healthy wood tissues are "trapped" between an older inner defect and a new wound. The new wound isolates the healthy wood from the cambium, the symplast is broken, and transport to the "trapped" wood is disrupted. Cells with living contents may die when branches or roots die.

Recent experiments show that the symplast may have an effect on the cambium. Holes were drilled through sugar maples, red maples, and paper birch. The holes ended approximately 0.5 cm from the cambium on the opposite side of the trees. Although the cambium on the opposite side of the hole was not touched, nor were there any cracks from the wound tip to the cambium, the growth rings distal to the end of the drill hole increased in width. The width of the growth rings attenuated tangentially away from the distal ends of the drill holes. It may be that the symplast plays a more important role than suspected in the regulation of the cambium. The cambium may be like a queen bee, it only produces cells. The differentiation of the cell may be determined by other conditions. When a hive is in trouble, more soldiers develop. After tree injury or infection, a barrier zone develops.

The barrier zone is a key boundary in trees (Tippett and Shigo 1980, Mulhern et al. 1979). The zone is a nonconducting tissue that has a few vessels in deciduous hardwoods and an abundance of axial and ray parenchyma. The cell walls have low amounts of lignin. In oaks, suberin was in the cell walls (Pearce and Rutherford 1981). The barrier zone separates the tissues present at the time of injury and infection from the few cells that continue to form after the zone is completed. In conifers, more resin ducts are formed in the barrier zone, and in eucalyptus species, more kino forms in the zone.

Within tissues present at the time of injury and infection, chemical boundaries are formed to resist spread of pathogens. Vessels begin to plug, and pits in tracheids close. Gums and other materials plug the vessels. Oxidation processes start. The contents in cells change to inhibitory materials. The changes serve to set chemical boundaries to resist spread of pathogens. The boundaries are the result of nonspecific reactions. The tree will react the same way for any number

of microorganisms or types of wounds. It is impossible to separate the effects of the wound from the microorganisms, and the wound and microorganisms from the tree on the formation of boundaries. The boundaries are the result of interactions between the tree and the pathogen after the trigger has been released by the wound.

The wound and microorganisms can kill cambium. When large areas of cambium are killed, that part of the tree dies. When more cambium is killed, the entire tree may die. Usually the killing does stop at some point. Then the first cells formed by the cambium differentiate to form a barrier zone. Under some conditions, aggressive microorganisms may grow through a barrier zone. If they do, it only occurs once because the cambium will be killed. A barrier zone will also form as a response to infection. For example, as Ceratocystis ulmi grows through a vessel, it does not contact the cambium, but a barrier zone may form.

Little is known about the factors that affect formation and size of the barrier zone. Small wounds may elicit large barrier zones, and large wounds may have small zones. Sometimes the zones develop completely around the trunk, while most of the time they do not. Barrier zones form in roots also.

ENERGY NEEDS

Energy is required to run the biological machinery. Trees store energy as starch or oils in living cells. It is one thing to trap energy, and another to have some place to store it. When compartmentalization walls off too much tissue, the process can be counterproductive. Trees have evolved in such a way that the volume of storage space is a basic feature of different species of trees. For example, American elms store starch in 15 to 18 growth rings. When elms infected by C. ulmi begin compartmentalizing the pathogen, portions of the tree are reduced to a one-growth-ring tree. The fungus is well adapted to killing one-growth-ring branches or trees.

When a tree is two years old, all the living cells in the wood can store energy. As the tree gets older, and as living cells die, the ratio of the volume of wood in the stem to the volume of wood that can store energy reserves decreases. This is a part of the aging process.

Biological aging is the orderly, genetically controlled change in a part, or the entire organism, as highly ordered processes deteriorate. Aging is an intrinsically controlled process. The changes are set in the genetic program, but environmental factors affect the expression of the changes. The different types of wood cells age at different rates. All cells start as cambium and then begin to differentiate. Vessels lose their contents and become functional for transport. Vessels age rapidly to the point of function. Fibers may contain living contents for a few months or for several years. Ray and axial parenchyma may maintain living contents for over a hundred years. The entire rhythm of changes built into one growth ring repeats as a new growth ring is formed.

Biological decaying is the interaction of intrinsic and extrinsic factors that affect changes in parts or the entire organism as high order goes to low order, and energy is transferred from the host to the pathogen. Decaying involves intrinsic and extrinsic factors, and there is an energy transfer from tree to pathogen that is detrimental to the tree.

As cells age and as new growth rings envelop older rings, the aging cells are buried deeper. The young growth rings with the high percentage of living cells can respond rapidly and effectively to injuries and infections. But, as wounds go deeper into the wood, the older, aged cells are exposed to the pathogens. If trees did not have some way to keep the microorganisms from quickly digesting the aging cells, trees would not exist today.

Some aged cells contain extractives that stall the growth of microorganisms. But in spite of all of the tree's protection and defense adaptations, the microorganisms do win at times, and wood is digested. Hollows are common in old trees.

CODIT

Compartmentalization is a boundary-setting process and CODIT is a model of the process. CODIT is an acronym for Compartmentalization Of Decay In Trees. CODIT has two parts: Part I is represented by 3 model walls and Part II by one wall. In Part I, wall 1 resists--not stops--vertical spread of pathogens, wall 2 resists inward spread, and wall 3 resists lateral or tangential spread. After injury and infection, Part II or wall 4 is formed and separates Part I from the healthy cells that continue to form in a new spatial position. Wall 4 is a model representative of the barrier zone. The CODIT model should not be identified too closely with anatomical or biochemical features. It is a model to help orient the mind to the three-dimensional spread of the pathogen and to show the separation between infected cells and the cells that continue to form.

When CODIT is used to reexamine tree problems, the orderly patterns of spread and resistance to spread emerge. CODIT shows that the spread of microorganisms is orderly, and so is the response of the tree.

CANKER AND CANKER ROTS

Cankers are localized lesions or dead spots. Cankers show the intimate interaction of tree and pathogen.

Pathogens infect trees four ways: 1) infect in bark and stay in bark; 2) infect in bark, grow into wood; 3) infect in wood, stay in wood; 4) infect in wood, grow into bark. The pathogens that stay in the wood are typical "wound rots." Those that stay in bark and may grow slightly into the wood, are those that cause annual cankers. The pathogens that grow from wood to bark, or bark to wood, start a seesaw interaction with the tree. In such interactions, it seems that compartmentalization is not functioning, and that is correct for short periods. Compartmentalization is intermittent. If compartmentalization were not effective at all, the tree would die.

Some microorganisms infect bark and continue to grow until the tree either forms a boundary in the bark, or the tree forms a wood wedge from xylem into the bark. The ray sheets that extend from xylem to phloem may form wedges of wood in front of the advancing pathogen in the bark. The wood wedges form only up to the phellem. When the pathogen begins to grow again, it usually expands into the phellem around the wood wedge and then back down into the bark. The tree then forms another wood wedge, and the process is repeated. Wood wedges are formed by oaks attacked by Strumella coryneoidea, and aspens attacked by Hypoxylon mammatum.

Fungi that cause canker rots get established in wood and then produce wedges of hard fungus material into the bark. The fungus wedge expands in the bark and kills cambium from the bark side. When the cambium is killed, the still-living cambium beyond the dead spot responds to begin compartmentalization again--another seesaw interaction.

In some American chestnut trees, pillars of xylem ray parenchyma burst into the dying bark and a new cambium is generated, and wood and bark continue to form.

Trees have marvelous ways of staying alive.

LIMITS OF COMPARTMENTALIZATION

Compartmentalization, like healing, has its limits. Here are some common examples where boundaries are broken and the pathogens spread rapidly.

1. Cracks that split outward from wounds--so called "frost cracks".
2. Insects and animals that rupture the barrier zones from the inside.
3. Barrier zone broken during cleaning prior to cavity filling.
4. Holes drilled through barrier zones.
5. Perennial cankers and canker rots--temporary seesaw interaction.
6. Aging. Given enough time, all natural materials will break down.
7. Energy depletion. When energy reserves are so reduced, inhibitory materials may not form.

TREES RESISTANT TO SPREAD OF DECAY

The tree protection features and defense systems when operating properly, make it possible for trees to survive for hundreds or even thousands of years. Some individuals in a species have much more effective protection features and defense systems than others. Recent research shows that compartmentalization is under moderate to strong genetic control (Lowerts and Kellison 1981). This means that the strong individuals can be selected for seeds or cuttings. Indeed, it is possible to select trees resistant to spread of decay.

GROUP SURVIVAL

What has been discussed to this point deals with protection and defense of the individual trees against many microorganisms and the environment. Trees must also have group protection and defense against pathogens and environmental pressures.

Some of the group protection features may be gregarious growth habits, great diversity in gene pool, and asynchronous phenology--factors that prevent selfing. By this type of reasoning, trees must also have a group defense system. It is difficult to go beyond conjecture here, but some of the group dynamic defense systems may include volatile chemicals that signal possible injury to the group and changes in some electrical type signals that may alert the group to react to change their physiology in favor of defense. Enough said here. There has been too much said already on this subject and not enough sound work done to support the statements.

SUMMARY

We do not have all the answers about trees. We must go back and reexamine many parts of tree biology. I am sure there are still many surprises.

Trees and tree inhabiting organisms do play a time game with the ever-changing environment. Trees survive so long as they have time to generate enough new cells to maintain the tree after injury and infection. The new cells must not only store energy reserves and carry out the normal tree physiological functions, but also they must maintain mechanical support. While the new cells are being faced with these responsibilities, the older cells that were present at the time of injury and infection must resist, stall, or limit the spread of the pathogens. If the pathogens spread faster than the newly generated cells can take over, the tree part or the entire tree may die.

Trees have protection features and a defense system. The defense system is centered about forming new boundaries or reinforcing existing boundaries. After injury and infection, the tree can respond or react only within its genetically set anatomy and physiology. It can only form so many inhibitory compounds, and it can alter its anatomy only so much. Some of the changes that are made are similar to the built-in protection features. But, a tree cannot overplug its transport system or use too much of its stored reserves for boundary materials without putting a strain on its health.

What keeps a tree alive eventually kills it. It is a time game against environmental factors and the many other organisms that want its space and energy. But so long as some trees can continue to play the game as they have for hundreds of millions of years, trees will survive.

LITERATURE CITED

Boyce, J.S. 1961. Forest Pathology. 3rd ed. 572 p. NY. McGraw-Hill.

Lowerts, G.A., and Kellison, R.C. 1981. Genetically controlled resistance to discoloration and decay in wounded trees of yellow poplar. Silvae Genetica 30: 98-101.

Mayer-Wegelin, H. 1936. Astung. 178 p. Hannover: M & H. Schaper.

Mulhern, J., Shortle, W.C., and Shigo, A.L. 1979. Barrier zones in red maple: An optical and scanning microscope examination. Forest Sci. 25:311-316.

Pearce, R.B., and Rutherford, J. 1981. A wound associated suberized barrier to the spread of decay in sapwood of oak (Quercus robur L.). Physiol. Plant Pathol. 19: 359-369.

Shigo, A.L. 1983. Tree defects: A photo guide. USDA For. Serv. Gen. Tech. Rep. NE-82. 167 p. Northeast. For. Exp. Stn., Broomall, PA.

Shigo, A.L. 1984. Compartmentalization: A conceptual framework for understanding how trees grow and defend themselves. Ann. Rev. Phytopathol. 22:189-214.

Shigo, A.L., and Marx, H. 1977. Compartmentalization of decay in trees. (CODIT). U.S. Dep. Agric. Inf. Bull. 405. 73 p.

Shigo, A.L., and Shortle, W.C. 1983. Wound dressings: Results of studies over 13 years. J. Arboric. 9:317-329.

Shigo, A.L., and Shortle, W.C. 1985. Shigometry. U.S. Dep. Agric. Hdbk. (In press).

Tippett, J.T., and Shigo, A.L. 1980. Barrier zone anatomy in red pine roots invaded by Heterobasidion annosum. Can. J. For. Res. 10:224-232.

Freezing Injury in Woody Tissues of Peach

E. N. Ashworth
USDA, Appalachian Fruit Research Station
Kearneysville, West Virginia

Winter injury is a predisposing factor in the development of Cytospora canker and subsequent tree decline. Injury from low winter temperatures provides entry ports for the fungus (6,10,12,18) and reduces the tree's subsequent ability to resist the spread of the pathogen (12). The purpose of this brief review is to describe the freezing process in peach trees and discuss the current concepts on the mechanism of injury.

Intact trees supercool very little and ice formation is initiated between -0.6 and -2.6 C (2,4). Ice formation appears to be initiated at several locations within the tree and subsequently spreads throughout. On the average, tissues freeze above -2 C. The rate at which ice spreads throughout a tree is variable. We have observed ice to spread throughout a mature tree growing in the orchard within 16 minutes, however, time intervals longer than one hour have also been observed. The time required for ice to spread throughout the tree is a function of the prevailing weather conditions, mass, and the extent of supercooling prior to freezing (4).

As ice forms within the tree, the release of the heat of fusion warms the tissue relative to air temperature. The rate at which the heat of fusion is dissipated depends on the prevailing weather conditions, the mass of the tissue, and the rate of heat transfer. Large tissues, such as the trunk and scaffold limbs, can remain 2 to 3 C warmer than air temperature for several hours after freezing has been initiated. We have observed that on many evenings trunk temperature never reaches the low air temperature. Whether this influences tissue survival under some conditions is not known.

The response of bark and xylem tissues to a freezing stress is quite different at the cellular level (1,5,7,8,15). In bark, ice crystals begin to form in the intercellular spaces. As freezing progresses, the vapor pressure of the extracellular water declines and water diffuses through the membrane to the intercellular space in order to reestablish equilibrium. The net result is that as temperature declines, the liquid water content of the cell decreases markedly (5,13). Therefore, during freezing, cells undergo dehydration. This in turn results in a reduction in cell volume and surface area, a decrease in the hydration of structural components and macromolecules, and an increase in cell osmolality (13). Although it is easy to deduce the numerous stresses which would occur during freezing, it is not clear which, if any, are responsible for freezing injury. Although it is still not clear how tissues are injured by freezing, it appears that the cell membrane is the site of injury. Early symptoms of freezing injury include the loss of both selective permeability and osmotic responsiveness (5,13). The cold hardiness of bark tissue is a function of the cell's ability to tolerate the numerous stresses which accompany freezing. Since tissue water freezes over the same temperature range throughout the year, acclimation in the fall and deacclimation in the spring reflect seasonal changes in the ability to tolerate extra- cellular freezing and the accompanying stresses (1).

The xylem parenchyma cells of peach behave like those of many other deciduous hardwoods and avoid freezing injury by deep supercooling (1,5,7,8,14,15,16). Differential thermal analysis of peach xylem reveals two distinct exotherms upon freezing. The first corresponds to the freezing of water within the xylem vessel

elements. The second low temperature exotherm corresponds with the killing point of the tissue and is believed to result from intracellular ice formation within the xylem parenchyma cells (1,5,7,8,11,15).

The temperature of the low temperature exotherm varies seasonally. It appears that acclimation and deacclimation reflect changes in the extent of supercooling by these tissues. It is not clear what causes changes in the extent of supercooling or how acclimation and deacclimation are controlled.

The freezing behavior of xylem parenchyma cells is similar to that observed when small water droplets are dispersed in oil (17). Water droplets supercool to temperatures approaching the homogeneous nucleation temperature (-38 C) of water. Once freezing was initiated within a droplet, the entire droplet froze rapidly, but ice was unable to spread to adjacent droplets. Similarly, xylem parenchyma cells appear to freeze as individuals or small groups of cells and the proportion of cells killed corresponds to the amount of supercooled water which was frozen (1,11).

Exactly which properties of hardwood tissues enable cells to deep supercool has not been determined. Some feature of the tissue must prevent ice from spreading throughout the tissue and also prevent the sublimation of supercooled water to ice in adjacent tissues. Structural features of the tissue appear to be important in deep supercooling (1,7,8,14,16) and several proposals have been advanced to account for their role (3,7,8,16).

Some recent observations have altered our concept of how xylem parenchyma cells respond to a freezing stress. Gusta and coworkers (9) investigated deciduous hardwood species which have been observed to supercool yet live north of the -40 C isotherm. When tissues from these species are exposed to low temperatures for extended periods, the low temperature exotherm decreases in size and occurs at lower temperatures. In some instances, a complete disappearance of the low temperature exotherm was observed (9). These results implied that the xylem parenchyma cells dehydrate in response to the extracellular ice and do not behave as isolated water droplets. In addition, recent work in our laboratory has demonstrated that the plasmalemma of peach is responding to a dehydrative stress. Both internal and external vesiculation and deep invaginations of the membrane were noted when the xylem parenchyma cells of peach were exposed to a freezing stress (19). Therefore, it appears that the behavior of these cells and the mechanism of injury is not as straightforward as previously thought and further work will be required to elucidate the mechanism of injury.

Although considerable progress has been made in understanding how plants respond to freezing, there are many unanswered questions. In addition, we need to know not only how tissues respond to freezing stress but how injured tissues respond towards a pathogen. Even within the same tree, injury will occur over a range of temperatures, therefore, it would be quite common for a mixture of dead, injured, and normal cells to be observed after a freezing stress. How such tissues respond when challenged by a pathogen is not known and remains an important area for future research.

Literature Cited

1. Ashworth, E. N., D. J. Rowse, and L. A. Billmyer. 1983. The freezing of water in woody tissues of apricot and peach and the relationship to freezing injury. J. Amer. Soc. Hort. Sci. 108:299-303.

2. Ashworth, E. N. and G. A. Davis. 1984. Ice nucleation within peach trees. J. Amer. Soc. Hort. Sci. 109:198-201.

3. Ashworth, E. N. and F. B. Abeles. 1984. Freezing behavior of water in small pores and the possible role in the freezing of plant tissues. Plant Physiology 76:201-204.

4. Ashworth, E. N., J. A. Anderson, G. A. Davis, and G. W. Lightner. 1984. Ice formation in Prunus persica under field conditions. J. Amer. Soc. Hort. Sci. (Submitted)

5. Burke, M. J., L. V. Gusta, H. A. Quamme, C. J. Weiser, and P. H. Li. 1976. Freezing injury in plants. Annu. Rev. Plant Physiol. 27:507-528.

6. Dhanvantari, B. N. 1978. Cold predisposition of dormant peach twigs to nodal cankers caused by Leucostoma spp. Phytopathology 68:1779- 1783.

7. George, M. F. and M. J. Burke. 1977. Cold hardiness and deep supercooling in xylem of shagbark hickory. Plant Physiol. 59:319- 325.

8. George, M. F. 1983. Freezing avoidance by deep supercooling in woody plant xylem: Preliminary data on the importance of cell wall porosity. In D. D. Randall, D. G. Blevins, R. L. Larson, and D. J. Rapp (Eds.) Current Topics in Plant Biochemistry and Physiology. Univ. of Missouri Press. pp. 84-95.

9. Gusta, L. V., N. J. Tyler, and T. H. Chen. 1983. Deep undercooling in woody taxa growing north of the -40 C isotherm. Plant Physiol. 72:122-128.

10. Helton, A. W. 1961. Low temperature injury as a contributing factor in Cytospora invasion of plum trees. Plant Dis. Rep. 45:591-597.

11. Hongs, S. G. and E. Sucoff. 1980. Units of freezing of deep supercooled water in woody xylem. Plant Physiol. 66:40-45.

12. Gable, P. F., P. Fliegel, and K. G. Parker. 1967. Cytospora canker on sweet cherry in New York State: Association with winter injury and pathogenicity to other species. Plant Dis. Rep. 51:155-157.

13. Levitt, J. 1980. Responses of Plant to Environmental Stresses. Vol. 1, Academic Press, New York.

14. Quamme, H. A., C. Stushnoff, and C. J. Weiser. 1972. The relationship of exotherms to cold injury in apple stem tissues. J. Amer. Soc. Hort. Sci. 97:608-613.

15. Quamme, H. A., C. J. Weiser, and C. Stushnoff. 1973. The mechanism of freezing injury in xylem of winter apple twigs. Plant Physiol. 51:273-277.

16. Quamme, H. A., P. M. Chen, and L. V. Gusta. 1982. Relationship of deep supercooling and dehydration resistance to freezing injury in dormant stem tissues of 'Starkrimson Delicious' apple and 'Siberian C' peach. J. Amer. Soc. Hort. Sci. 107:299-304.

17. Rasmussen, D. H. and A. P. Mackenzie. 1973. Clustering in supercooled water. J. Chem. Phys. 59:5003-5013.

18. Willison, R. S. 1937. Peach canker investigation. III. Further notes on incidence contributing factors and related phenomena. Can. J. Res., Sect. Biol. Sci. C. 15:324-339.

19. Wisniewski, M. E. and E. N. Ashworth. 1984. Changes in the ultrastructure of xylem parenchyma cells of peach [Prunus persica (L.) Batsch] and Red Oak [Quercus rubra (L.)] in response to a freezing stress. Amer. J. Bot. (Submitted)

POTENTIAL FOR BIOLOGICAL CONTROL OF *CYTOSPORA* CANKER ON STONE FRUITS

James A. Traquair
Agriculture Canada, Research Station
Harrow, Ontario, Canada NOR 1GO

Introduction

Lack of an easy, "one-shot" chemical remedy has made the control of Cytospora canker very frustrating for peach growers. The question arises as to the possibilities of an alternative control involving biological agents. In actual fact, the value of biological materials in curing plant diseases such as cankers has been recognized for many years. This approach which is currently receiving intensive research interest, entails the introduction of micro-organisms that antagonize the canker-causing fungus or the enrichment of populations of canker epiphytes already established on the bark. Common mechanisms of biological control include competition for space and nutrients, production of chemicals by the antagonist that kill the fungal pathogen, and direct invasion of the pathogen by another microbe. Other mechanisms include predation by microfauna and hypovirulence by virus-like agents.

At Harrow Research Station, several fungal antagonists have been isolated from the surface of and within old cankers and have been shown to inhibit the canker-causing fungus under artificial conditions. Our aim is to eradicate the perennial phase of the canker on the trunk and major branches by manipulating environmental conditions that encourage natural antagonists already associated with canker in the orchard. The challenge is to integrate biological control measures with routine orchard management practices which include pesticide sprays and pruning.

Stone fruits, particularly peaches and nectarines, are very susceptible to perennial canker, a ubiquitous fungal disease caused by *Leucostoma cincta* (Pers. ex Fr.) Höhn. (imperfect state = *Cytospora cincta* Sacc.) and *L. persoonii* (Nits.) Höhn. (imperfect state = *C. leucostoma* Sacc.). These fungi are facultative parasites and are often considered to be secondary invaders of unhealed leaf scars, fruit pedicels, buds or wood damaged by cold, pruning, feeding insects and other pathogens. Infection leads to necrosis and the development of depressed, gummy, perennial cankers that continue to enlarge as the pathogen advances through infected woody tissues. Girdling of branches results in wilting and dieback symptoms. Cytospora canker is, therefore, a conspicuous disease. The complexity of predisposing and interacting factors has confounded estimation of damage by *Cytospora* spp. alone and has impeded attempts to control the disease. Paramount among these factors are lack of cold hardiness and general orchard mismanagement.

Our research at Harrow is aimed primarily at the control of the perennial phase of peach canker. In southern Ontario, *C. leucostoma* is isolated frequently (29) but *C. cincta* is the primary cause of twig dieback (71).

Control strategies aimed at alleviating the incidence and severity of perennial canker by reducing levels of inoculum in the orchard and by minimizing the influence of predisposing factors are not completely effective. Nevertheless, orchard site, choice of cultivars, tillage, fertilization, pruning, and pest control in established orchards are important factors in the 10-point control recommendations (14). These control measures are often time consuming, labor intensive and costly. Fungicidal protection of potential infection courts has been somewhat effective but the chemical eradication of *Cytospora* from established cankers has not. The chances

of finding an economical, "one-shot" chemical control for canker seem remote. The nature of the crop and complexity of predisposing factors make the breeding of resistant stone fruits very slow and difficult. Host immunity to perennial canker has not been found but varied resistance or tolerance to canker in the field and artificial environments has been observed (64). Early detection of an intrinsic resistance mechanism is required to provide protection during the dormant and growing periods.

Studies of Cytospora antagonists in Canada, the United States and Europe have indicated a potential for the microbial control of perennial canker. Preventative treatments with biological agents (such as bacteria and fungi) have been effective while eradicative measures have not. Excellent results have been obtained in the use of virus-like factors from hypovirulent strains of Endothia parasitica to eradicate chestnut blight and canker in Europe and the United States. Similar hypovirulence factors may exist in Cytospora spp. for which variability in pathogenicity has been reported. Biological control of the overwintering mycelium in perennial cankers would fit well in an integrated management scheme.

Biological control in the aerial portions of trees

Writings dated as early as 1675 describe the use of biological materials containing microorganisms to protect apple trees against canker diseases (24). Early horticulturists recommended the washing of fresh pruning wounds with cow dung and urine followed by the application of plasters made by mixing horse manure and clay. Scientists are now evaluating specific microbial components of soil such as Bacillus subtilis and various Hyphomycetes, including Trichoderma and Fusarium species to protect pruning wounds from wood-decay and canker-causing fungi (48, 67). In fact the use of Fusarium lateritium and benomyl on pruning wounds is very successful as an integrated management approach to the control of Eutypa canker in apricot trees in Australia (18, 19, 45).

Biological balance and management of the agroecosystem are important considerations in the development of a successful and stable biological control of plant diseases (22, 23). Host plant resistance and manipulation of cultural practices have traditionally been well-established mechanisms of biological control. However, for purposes of this review, biocontrol methods will be restricted to the encouragement or introduction of microorganisms that are antagonistic to plant pathogens. Unfortunately, lack of information on the ecology and conditions that promote antagonism, has frequently hindered the demonstration of practical biological control under field conditions (5).

The development of biological control strategies have been slow and most of the interest has centered around antagonism of soil-borne plant pathogens (48). Less research has been undertaken in the biological control of parasites in the aerial portions of plants. Of 78 projects identified, only four registered biocontrol strategies are used on a commercial scale (48). Two of these involve tree pathogens, ie. the biological control of crown gall (Agrobacterium tumefaciens) in tree fruits with A. radiobacter (43, 44, 52, 28) and the control of Fomes annosus decay in pines with Peniophora gigantea (61). Early pessimism about biological control is gradually being dispelled by recent reports of success in specific field situations.

Biological control must involve the reduction of pathogen inoculum and the protection of plant surfaces that serve as infection sites (7, 21). Both objectives can be accomplished through competition with the plant pathogen, antibiosis,

antagonistic bacteria (fluorescent Pseudomonads), Aureobasidium pullulans, Cladosporum and Fusarium spp. (16). In addition, sublethal dosages of methyl bromide applied to orchard soils are reported to allow Trichoderma viride to replace Armillaria mellea which causes root rot of citrus trees (54).

Research into the manipulation of micro-organisms in the phylloplane (leaf surfaces) and the caulosphere (stem surfaces), is not well advanced. A better understanding of the ecology of antagonists and plant pathogens on these surfaces is urgently required. Early research on tree fruit epiphytes has shown that a variety of filamentous fungi (e.g. Coniothyrium, Cladosporium, Fumago, Fusarium, Alternaria, Phoma, Sporotrichum) and yeasts occur as saprophytes on buds and bark (46, 47). Recent studies of beech (Fagus) bark epiphytes show that Alternaria, Aureobasidium, Cladosporium and Epicoccum are very abundant (26). These epiphytes are also found on peach buds and bark (62). Many of these innocuous fungi have thick, darkly pigmented cells that resist drying, low temperatures and ultra-violet light. As ubiquitous epiphytes they play a dominant role in the microbial ecology of the aerial parts of most plants (68, 58). As strong competitors for space and nutrients, they are able to crowd out plant pathogens under appropriate conditions (33, 69, 25, 57, 11).

Introduction of antagonists

A traditional approach to biological control is the introduction of non-resident micro-organisms or the restoration of indigenous antagonists in the ecosystem. Unfortunately, the mechanism of antagonism is rarely studied in depth and often interactions are only known from experiments in vitro (5). Mechanisms include antibiosis, predation and direct hyperparasitism (mycoparasitism). Much work is required to determine the ecological requirements that promote antagonistic reactions with plant pathogens in field situations. Important considerations are rate of spread of the antagonist, speed of attack, potential for debilitation, specificity and persistence (50). Many approaches have been attempted in the biological control of tree pathogens and some practical success has been reported in the use of biological agents to protect infection sites such as pruning wounds (24).

1) Antibiosis. In a narrow sense, antibiosis is the antagonism between two organisms resulting in one overcoming the other by the production of a growth inhibitory substance (antibiotic). This phenomenon is common for soil-borne microorganisms and is characterized by lysis of cell walls, the coagulation and disorganization of cytoplasm, and the distortion or death of cells. Antibiotic production is frequently attributed to bacteria, especially filamentous types (actinomycetes), but, many fungi have been reported to produce inhibitory substances in natural and artificial environments (7).

In aerial environments, particularly bark and wood, antibiotic-producing bacteria have been observed. Antagonistic Bacillus spp. and several Streptomyces spp. have been reported in the wood of apple (12), maple (13) and beech trees (26). In fact, Wensley (75) in his studies of peach bark epiphytes attributed the reduced incidence of Cytospora canker to increased populations of antagonistic actinomycetes.

Many different fungi in wood and bark environments produce antibiotic substances. For example, Ascocoryne sarcoides colonizes conifer wounds at an early stage of fungal succession and inhibits growth of wood-decay basidiomycetes (10). At the same time, some wood-decay basidiomycetes have a competitive advantage in being able to produce antibiotics (71). The production of such compounds by Peniophora gigantea has been shown to be a means by which this basidiomycete prevents infection

of stumps by the decay fungus and heart rot pathogen, Fomes annosus, which spreads in the roots of trees (41). This interaction has formed the basis for practical biological control procedures in Britain (24, 60, 61). Recent results have shown that Trichoderma species (63) also inhibit Cytospora canker development. Trichoderma viride is a useful antagonist in the protection of pruning wounds of plum trees against silver leaf caused by Stereum purpureum (36), wounds of apple twigs against canker caused by Physalospora obtusa (51) and grapevines against dead arm disease caused by Phomopsis viticola (32). Similarly, Trichoderma species are reported to inhibit the development of Cytospora species in artifical culture and in wounded twigs under field conditions (66, 63). Other antagonists reported to inhibit Cytospora spp. causing peach canker include Alternaria alternata, Coniothyrium spp., Epicoccum purpurascens, and Talaromyces flavus (6, 62, 63). In most cases, these antagonists have greater potential as preventative than as curative (eradicative) biological controls of peach canker.

2) Predation. The feeding habits of microfauna on mycelium and spores of fungal pathogens in the aerial parts of plants need further investigation. Grazing by micro-arthropods (such as fungivorous mites) has been recorded on foliage and twigs (17). Arthropod feeding on blister rust of pine has been reported to result in a 10% reduction in aeciospore production in Alberta (56). In addition, nematodes (Aphelenchoides spp.) have been reported on pycnidia of Cytospora leucostoma where they were feeding on spores and hyphae (59). Selective feeding on Cytospora would be a possible biocontrol mechanism.

3) Hyperparasitism. The growth of one fungus on another is hyperparasitism or mycoparasitism in its broadest sense. An important distinction from antibiosis is contact by the host and parasite (9).

The biotrophic (balanced) mycoparasite obtains nutrients from living cells which it contacts by means of simple, undifferentiated hyphal branches or by specialized penetration structures that give rise to absorptive branches (haustoria) in the host cell. Examples of biotrophic mycoparasites that attack canker-causing, fungal plant pathogens of twigs include Calcarisporium parasiticum on Physalospora obtusa (8, 39) and Gonatorrhodiella highlei on Nectria coccinea (34, 65, 31).

The necrotrophic (destructive) hyperparasite, on the other hand, utilizes nutrients released from host cells that have been killed by toxic substances excreted from contacting parasite hyphae. Penetration of the host may or may not occur and the antagonist essentially exists as a saprophyte. Often, these hyperparasites produce strong antibiotics. The examples of necrotrophic mycoparasites are numerous. Trichoderma spp. are effective parasites of many fungal pathogens, particularly wound parasites of trees and have been discussed with regard to production of antibiotic substances. T. viride parasitizes the silver leaf pathogen of pome and stone fruits (36). T. harzanium is an important hyperparasite of the white-rot basidiomycete, Coriolus versicolor, which enters wounds on maple trees (55). In fact, numerous wood-decay basidiomycetes such as C. versicolor, Bjerkandra adusta, and Hirschioporus pargamenus are hyperparasites themselves (35, 51).

Necrotrophic mycoparasites are of considerable interest for their eradicative biocontrol of systemic obligate parasites such as the pine gall rust, Endocronartium harknessii, parastized by Cladosporum gallicola (73) or pine blister rust, Cronartium ribicola, parasitized by Tubercularia maxima (49, 76). Similar parasites would be useful in the control of gall- or perennial canker-forming, facultative pathogens of tree fruits. Early studies by Koch (46, 47) in the Niagara region of Ontario pointed to the biological control potential of fungi such as Cephalothecium roseum, Coniothyrium, Hendersonula, Sphaeronema, and Cladosporium spp., which he

found parasitizing the stromatic reproductive structures of Dibotryon morbosum and reducing inoculum levels for plum and cherry knot. Fusarium lateritium has already been shown to be an effective hyperparasite of Eutypa armeniacae which causes dieback and perennial canker of apricots in Australia (18). Preliminary experiments in the biocontrol of twig and nodal canker in peach trees with Trichoderma spp., Coniothyrium spp., Epicoccum purpurascens, and Talaromyces flavus have shown some promise (6, 63).

4) Hypovirulence. A subnormal state of aggressiveness or pathogenicity is characteristic of hypovirulence (50). There are numerous causes for reduced virulence but plant pathologists are particularly interested in "contagious" or "infectious" hypovirulence as a source of biological control agents (50, 32). In this case, the infectious agent is a virus-like factor consisting of double stranded ribonucleic acid (dsRNA) which renders the fungal pathogen hypovirulent and spreads throughout the mycelium by anastomosis. This factor is borne in the fungal cytoplasm and can be mechanically spread by insect vectors carrying infectious spores or by means of inoculation by man.

Many fungi contain dsRNA mycoviruses (40). Early demonstration of hypovirulence and the potential for biocontrol are illustrated by the report of declining virulence in Rhizoctonia solani which causes damping-off of sugar beets and cabbage (20). The observations in Italy by Biraghi and in France by Grente of natural regression of chestnut blight caused by Endothia parasitica (32) has led to practical, curative, biological control of this serious disease in Europe (2, 4). A similar hypovirulence was observed in North America where the etiology was traced to a virus-like factor (27). Recent, electron microscopy of hypovirulent strains from Europe have revealed club-shaped (30) and spherical virus particles (53). The hypovirulent strains of E. parasitica are readily recognized by unusual growth rate and color in culture (2). Unfortunately, problems with vegetative incompatibility between strains (1, 42) and lack of stability (3) has led to problems in the natural spread of the virus throughout American populations of E. parasitica (4).

The potential for exploiting hypovirulence in the eradicative control of Cytospora canker is unclear. There are reports of naturally-healed cankers (38) but the causes are unknown. Certainly, C. leucostoma, the predominant pathogen in perennial cankers, is a prime candidate for providing hypovirulent strains because varied pathogenicity is frequently reported for this species (37, 74, 77).

Prognosis

Reports of peach trees surviving for 50 years or more in China (14) suggest that there is considerable room for improvement in our current orchard management schemes. Pressure to improve yield and quality within the constraints of increased production costs has led to changes in cultivars and reduction in the labour intensive care of trees (e.g. canker surgery). Because of the complex epidemiology of perennial canker and short-life of peach trees, there is little reason to be optimistic in a search for a single, effective tactic. Biological control offers considerable potential but it must be incorporated in a multifactorial, integrated management strategy that takes into consideration sound ecological principles and concepts of ecosystems management (76).

Our research into the biological control of peach canker focuses on hypovirulence, the protection of pruning wounds and the eradication of Cytospora leucostoma from perennial lesions on the trunk and scaffold branches. We are aiming to manipulate epiphytes already established on cankers and peach bark. Towards this end, we have

isolated several yeast-like and filamentous fungi that inhibit the growth of C. cincta and C. leucostoma in artificial culture. These fungi include Sporobolomyces, Aureobasidium, Trichoderma, Gliocladium, Penicillium, Cladosporium, Chaetomium and Epicoccum species. Mechanisms of inibition based on observations of slide cultures include competition for nutrients, antibiosis and lysis, and/or direct hyphal invasion.

We are selecting potential biocontrol agents that tolerate lower temperatures. Then we will evaluate their activity and persistence under field situations in an attempt to integrate biological control with the routine spring and fall schedules of chemical spraying. Chemical control of diseases and insect pests, irrigation, fertilization, pruning, and cultivar selection (host resistance) must all be considered in order to reduce the impact of the diverse factors that predispose trees to canker development.

Literature Cited

1. Anagnostakis, S. L. 1977. Vegetative incompatibility in Endothia parasitica. Experimental Mycology 1: 306-316.

2. Anagnostakis, S. L. 1978. The American chestnut: new hope for a fallen giant. Conn. Agr. Expt. Sta. Bull. 777.

3. Anagnostakis, S. L. 1981. Stability of double-stranded RNA components of Endothia parasitica through transfer and subculture. Experimental Mycology 5: 236-242.

4. Anagnostakis, S. L. 1982. Biological control of chestnut blight. Science 215: 466-471.

5. Ayers, W. A., and Adams, P. B. 1981. Mycoparasitism and its application to biological control of plant diseases. In Biological Control and Crop Production. Edited by G. C. Papavizas. Allenheld, Osmun & Co., Publishers, Inc., Mount Claire, N.J. pp. 91-103.

6. Alm, G. R. and Patrick, Z. A. 1984. Microflora of peach leaf scars and its influence on peach canker caused by Leucosoma cincta (Abstract). Joint meeting of the Am. Phytopathol. Soc. and the Can. Phytopathol. Soc., August 1984, University of Guelph, Guelph, Ontario.

7. Baker, K. F., and Cook, R. J. 1974. Biological control of plant pathogens. W. H. Freeman and Co. Publishers, San Francisco.

8. Barnett, H. L. 1958. A new Calcarisporium parasitic on other fungi. Mycologia 50: 497-500.

9. Barnett, H. L., and Binder, F. L. 1973. The fungal host-parasite relationship. Annu. Rev. Phytopathol. 11: 273-292.

10. Basham, J. T. 1973. Heart rot of black spruce in Ontario. II. The mycoflora in defective and normal wood of living trees. Can. J. Bot. 51: 1379-1392.

11. Bier, J. E. 1965. Some inoculum factors in pathogenicity studies of Hypoxylon pruinatum (Klotzsche) Cke. on Populus tremuloides Michx. Can. J. Bot. 43: 877-883.

12. Blanchette, R. A. 1979. A study of the progressive stages of discoloration and decay in Malus using scanning electron microscopy. Can. J. For. Res. 9: 464-469.

13. Blanchette, R. A., Sutherland, J. B., and Crawford, D. L. 1981. Actinomycetes in discolored wood of living silver maple. Can. J. Bot. 59: 1-7.

14. Brittain, J. A., and Miller, R. W. 1978. Managing peach tree short life in the Southeast. Agric. Ext. Serv. of Ga., Ala., N.C., S.C., and U.S.D.A., Circ. 585. 19 pp.

15. Broadbent, P., and Baker, K. F. 1975. Soils suppressive to Phytophthora root rot in Eastern Australia. In Biology and control of soil-borne plant pathogens. Edited by G. W. Bruehl. Am. Phytopathol. Soc., St. Paul, Minn. pp. 152-157.

16. Burchill, R. T., and Cook, R. T. A. 1971. The interaction of urea and microorganisms in suppressing the development of perithecia of Venturia inaequalis (Cke.) Wint. In Ecology of leaf surface micro-organisms. Edited by T. F. Preece and C. H. Dickinson. Academic Press Inc., New York, N.Y. pp. 471-483

17. Carroll, G. C. 1981. Microbial productivity on aerial plant surfaces. In Microbial ecology of the phylloplane. Edited by J. P. Blakeman. Academic Press Inc., London, U. K. pp. 15-46.

18. Carter, M. V. 1971. Biological control of Eutypa armeniacae. Aust. J. Exp. Agric. Anim. Husb. 11: 687-692.

19. Carter, M. V., and Price, T. V. 1975. Biological control of Eutypa armeniacae. III. A comparison of chemical, biological and integrated control. Aust. J. Agric. Res. 26: 537-543.

20. Castanho, B., and Butler, E. E. 1978. Rhizoctonia decline: studies on hypovirulence and potential use in biological control. Phytopathol. 68: 1511-1514.

21. Cook, R. J. 1977. Management of the associated microbiota. In Plant Disease. An advanced treatise Vol. 1. How disease is managed. Edited by J. G. Horsfall and E. B. Cowling. Academic Press, Inc., New York, N. Y. pp. 145-166.

22. Cook, R. J. 1981. Biological control of plant pathogens: overview. In Biological control and crop production. Edited by G. C. Papavizas. Allenheld, Osmun, & Co. Publishers, Inc., Mt. Claire, N.J. pp. 471-483.

23. Cook, R. J. and Baker, K. F. 1983. The nature and practice of biological control of plant pathogens. The Am. Phytopathol. Soc., St. Paul, Minnesota. 539 pp.

24. Corke, A. T. K. 1978. Microbial antagonisms affecting tree diseases. Ann. Appl. Biol. 89: 89-114.

25. Corke, A. T. K., and Hunter, T. 1979. Biocontrol of Nectria galligena infection of pruning wounds on apple shoots. J. Hortic. Sci. 54: 47-55.

26. Cotter, H. Van T., and Blanchard, R. O. 1982. The fungal flora of bark of Fagus grandifolia. Mycologia 74: 836-843.

27. Day, P. R., Dodds, J. A., Elliston, J. E., Jaynes, R. A., and Anagnostakis, S. L. 1977. Double-stranded RNA in Endothia parasitica. Phytopathol. 67: 1393-1396.

28. Dhanvantari, B. N. 1976. Biological control of crown gall of peach in southwestern Ontario. Plant Dis. Reptr. 60: 549-551.

29. Dhanvantari, B. N. 1982. Relative importance of Leucostoma cincta and L. persoonii in perennial canker of peach in southwestern Ontario. Can. J. Plant Pathol. 4: 221-225.

30. Dodds, J. A. 1980. Association of type 1 viral-like ds RNA with club-shaped particles in hypovirulent strains of Endothia parasitica. Virology 107: 1-12.

31. Dubos, B., and Bulit, J. 1981. Filamentous fungi as biocontrol agents on aerial plant surfaces. In Microbial ecology of the phylloplane. Edited by J. P. Blakeman. Academic Press Inc., London, U.K. pp. 353-367.

32. Dubos, B., Bulit, J., Bugaret, Y., and Verdu, D. 1978. Possibilités d'utilisation de Trichoderma viride Pers. comme moyen biologique de lutte contre la pourriture grise (Botrytis cinerea Pers.) et l'excoriose (Phomopsis viticola Sacc.) de la vigne. C. R. Acad. Agric. Fr. 64: 1159-1168.

33. Fokkema, N. J., and J. W. Lorbeer. 1974. Interactions between Alternaria porri and the saprophytic mycoflora of onion leaves. Phytopathol. 64: 1128-1133.

34. Gain, R. E., and Barnett, H. L. 1970. Parasitism and axenic growth of Gonatorrhodiella highlei. Mycologia 62: 1122-1129.

35. Griffith, N. T., and Barnett, H. L. 1967. Mycoparasitism by basidiomycetes in culture. Mycologia, 59: 149-154.

36. Grosclaude, C., Ricard, J., and Dubos, B. 1973. Inoculation of Trichoderma viride spores via pruning shears for biological control of Stereum purpureum on plum tree wounds. Plant Dis. Reptr. 57: 25-28.

37. Helton, A. W., and Konicek, D. E. 1961. Effects of selected Cytospora isolates from stone fruits on certain fruit varieties. Phytopathol. 51: 152-157.

38. Hildebrand, E. M. 1947. Perennial canker and the canker complex in New York, with methods of control. Cornell Univ. Agric. Exp. Stn. Mem. 276. 61 pp.

39. Hoch, H. C. 1977. Mycoparasitic relationships. III. Parasitism of Physalospora obtusa by Calcarisporium parasiticum. Can. J. Bot. 55: 198-207.

40. Hollings, M. 1982. Mycoviruses and plant pathology. Plant Disease 66: 1106-1112.

41. Ikediugwu, F. E. O. 1976. The interface in hyphal interference by Peniophora gigantea against Heterobasidion annosum. Trans. Br. Mycol. Soc. 66: 291-296.

42. Jaynes, R. A., and Elliston, J. E. 1982. Hypovirulent isolates of Endothia parasitica associated with large American chestnut trees. Plant Disease 66: 769-772.

43. Kerr, A. 1974. Soil microbiological studies on Agrobacterium radiobacter and biological control of crown gall. Soil Sci. 118: 168-172.

44. Kerr, A. 1980. Biological control of crown gall through production of Agrocin-84. Plant Dis. 64: 24-30.

45. Klassen, W. 1981. The role of biological control in integrated pest management systems. In Biological control in crop production. Edited by G. C. Papavizas. Allanheld, Osmun & Co., Publishers, Inc., Mt. Claire, New Jersey. pp. 433-445.

46. Koch, L. W. 1934a. Studies on the overwintering of certain fungi parasitic and saprophytic on fruit trees. Can. J. Res. 11: 190-206.

47. Koch, L. W. 1934b. Investigation on black-knot of plums and cherries. II. The occurrence and significance of certain fungi found in association with Dibotryon morbosum (Sch.) T. & S. Sci. Agric. 15: 80-95.

48. Kommedahl, T., and Windels, C. E. 1981. Introduction of microbial antagonists to specific courts of infection: seeds, seedlings, and wounds. In Biological control in crop production. Edited by G. C. Papavizas. Allanheld, Osmun and Co. Publisher, Inc., Mt. Claire, N.J.pp. 227-248

49. Kranz, J. 1981. Hyperparasitism of biotrophic fungi. In Microbial ecology of the phylloplane. Edited by J. P. Blakeman. Academic Press, Inc., London, U. K. pp 327-352

50. Kuhlman, E. G. 1980. Hypovirulence and hyperparasitism. In Plant Disease, An advanced treatise. Vol. V. How plants defend themselves. Edited by J. G. Horsfall and E. G. Cowling. Academic Press, Inc., New York, N.Y. pp. 363-380.

51. Miller, P. M., and Anagnostakis, S. L. 1973. Piles of apple prunings as sources of conidia of Physalospora obtusa. Phytopathol. 63: 1080-1081.

52. Moore, L. W., and Warren, G. 1979. Agrobacterium radiobacter strain 84 and biological control of crown gall. Ann. Rev. Phytopathol. 17: 163-179.

53. Newhouse, J. R., Hoch, H. C., and MacDonald, W. L. 1983. The ultrastructure of Endothia parasitica. Comparison of a virulent with a hypovirulent isolate. Can. J. Bot. 61: 389-399.

54. Ohr, H. D., Munnecke, D. E., and Bricker, J. L. 1973. The interaction of Armillaria mellea and Trichoderma spp. as modified by methyl bromide. Phytopathol. 63: 965-973.

55. Pottle, H. W. Shigo, A. L., and Blanchard, R. O. 1977. Biological control of wound hymenomycetes by Trichoderma harzanium. Plant Dis. Reptr. 61: 687-690.

56. Powell, J. M. 1971. The arthropod fauna collected from the comandra blister rust, Cronartium comandrae, on lodgepole pine in Alberta. Can. Entomol. 103: 908-918.

57. Price, T. V. 1973. Studies on the microbial colonization of sapwood of pruned apricot trees. Aust. J. Biool. Sci. 26: 379-388.

58. Pugh, G. J. F., and Buckley, N. G. 1971. The leaf surface as a substrate for colonization by fungi. In Ecology of leaf surface micro-organisms. Edited by

T. F. Preece and C. H. Dickinson. Academic Press, Inc., New York, N.Y. pp. 431-445.

59. Pusey, P. L., and Slana, L. J. 1981. A nematode (Aphelenchoides) associated with Cytospora canker of peach. Phytopathol. 71: 770.

60. Rishbeth, J. 1963. Stump protection against Fomes annosus. III. Inoculation with Peniophora gigantea. Ann. Appl. Biol. 52: 63-77.

61. Rishbeth, J. 1975. Stump protection: A biological control of Fomes annosus. In Biology and control of soil-borne plant pathogens. Edited by G. W. Bruehl. Am. Phytopathol. Soc., St. Paul, Minn. pp. 158-162.

62. Royse, D. J., and Ries, S. M. 1978. Detection of Cytospora species in twig elements of peach and its relation to the incidence of perennial canker. Phytopathol. 68: 663-667.

63. Schulz, U. 1981. Untersuchungen zur biologischen Bekämpfung von Cytospora-Arten. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz. 87: 132-141.

64. Scorza, R. 1983. Resistance to Cytospora in stone fruit trees. Proc. 1982 Stone Fruit Decline Workshop, Michigan State University, East

65. Shigo, A. L. 1964. Organism interactions in beech bark disease. Symposium on cankers of forest trees. Phytopathol. 54: 263-269.

66. Smiley, E. T., Luepschen, N. S. and Newby, L. 1979. Trichoderma as a biological control agent for Cytospora canker. Colo. State Univ. Prog. Rep. 12.

67. Spurr, H. W. Jr., 1981. Introduction of microbial antagonists for the control of foliar plant pathogens. Pp. 323-332. In Biological control in crop production. Edited by G. C. Papavizas. Allenheld, Osmun and Co., Publishers, Inc. Mount Claire, N.J. pp. 323-332.

68. Stott, M. A. 1971. Studies on the physiology of some leaf saprophytes. In Ecology of leaf surface micro-organisms. Edited by T. F. Preece and C. H. Dickinson. Academic Press, Inc., New York, N.Y. pp. 203-210.

69. Swinburne, T. R. 1973. Microflora of apple leaf scars in relation to infection by Nectria galligena. Trans. Br. Mycol. Soc. 60: 389-403.

70. Tekauz, A., and Patrick, Z. A. 1974. The role of twig infections on the incidence of perennial canker of peach. Phytopathol. 64: 683-688.

71. Traquair, J. A., and McKeen, W. E. 1977. Hyphal interference by Trametes hispida. Can. J. Microbiol. 23: 1675-1682.

72. Traquair, J. A. and McKeen, W. E. 1978. Necrotrophic mycoparasitism of Ceratocystis fimbriata by Hirschioporus pargamenus (Polyporaceae). Can. J. Microbiol. 24: 869-874.

73. Tsuneda, A., and Hiratsuka, Y. 1979. Mode of parasitism of a mycoparasite, Cladosporium gallicola on western gall rust, Endocronartium harknessii. Can. J. Plant Pathol. 1: 31-36.

74. Wensley, R. N. 1964. Occurrence and pathogenicity of Valsa (Cytospora) species and other fungi associated with peach canker in southern Ontario. Can. J. Bot. 42: 841-857.

75. Wensley, R. N. 1971. The microflora of peach bark and its possible relation to perennial canker (Leucostoma cincta (Fr.) von Hohnel (Valsa cincta)). Can. J. Microbiol. 17: 333-337.

76. Wicker, E. F. 1981. Natural control of white pine blister rust by Tuberculina maxima. Phytopathol. 71: 997-1000.

77. Wysong, D. S., and L. E. Dickens. 1962. Variation in virulence of Valsa leucostoma. Plant Dis. Reptr. 46: 274-276.

INCIDENCE OF *CYTOSPORA* CANKER IN PENNSYLVANIA PEACH ORCHARDS: SURVEY RESULTS

James W. Travis and Kenneth D. Hickey
The Pennsylvania State University
University Park, PA 16802
and
The Fruit Research Laboratory
Biglerville, PA

Peach tree decline is perhaps the most important problem facing the Pennsylvania fruit industry. The causes of peach tree decline are varied and often indirect. To identify the causes of tree decline and to understand the complexes involved, a survey was conducted across the state in 1982 and 1983. This presentation will review the portion of the survey dealing with *Cytospora* canker. *Cytospora* canker is not only a cause of peach tree decline but often a result. In the survey, 50 sites, healthy and declining, were intensely studied to determine the relationship between canker and tree decline. Over 11,000 cankers were evaluated during the course of the survey. I'll present the information we obtained from the survey in the form of questions, which we asked ourselves about canker in Pennsylvania.

How much Cytospora canker is in the state?

Every one of the 50 sites had trees with canker. This is what we expected; there are not many orchards in the state that do not have some canker. I must emphasize that our observations were limited and so our findings are not representative of the whole Pennsylvania peach industry. The number of cankers observed on each site ranged from 4 to 1,546. The average number of cankers per tree was 23. In short, there is a lot of canker on peach trees in the state. Frankly, there were more cankers on each tree than we expected.

Are there more cankers on weak trees than healthy trees?

There was no difference in the number of cankers per tree on healthy and weak trees. On the good sites we found an average of 21 cankers per tree; on poor sites we found an average of 24 cankers per tree. But would we see a difference in canker if we consider different areas of the tree? On the average tree, there were 5 cankers on secondary branches, 16 on primary branches and 2 on the trunk. The average number of cankers in good trees on secondary branches was 5, primary branches - 14, and trunk - 2. On poor trees there was an average of 5 cankers on secondary branches, 17 on primary branches and 2 on the trunk.

Our conclusion; there is no real difference in the amount of canker between healthy and weak trees. However, we know that *Cytospora* only invades weakened trees. Perhaps the trees we classified as healthy trees were weakened at critical times during the year, which allowed *Cytospora* infections to begin. Certain factors may temporarily weaken trees such as; drought stress, a large crop, or pruning at the

wrong time. For instance, even a healthy tree will become infected with Cytospora if pruned before growth begins in the spring. So trees we consider as healthy may not be "healthy" all year long.

Can trees, especially "healthy" trees, heal canker wounds?

This is a very important question. Even if a tree is infected with Cytospora, if the infection can be "healed-out," the canker will not be a problem to the tree. It would be very important if healthy trees which were stressed and have canker could heal-out the infection. Healthy trees, for at least most of the season, would be the answer to our canker problems. We observed 11,283 cankers on the 500 trees that were a part of the survey. Of these, there was no healing response in 5 percent of the cankers, and there was a small healing response in 19 percent of the cankers. There was a good healing response in the majority of cankers but the healing was not complete. Only 3 percent of the cankers observed were completely healed. There was no difference in healing between healthy and weak trees. What have we learned from the survey results? In most cases, even weak trees exhibit a good healing response to Cytospora infection, but the healing is not usually complete.

What kind of wounds are most important to new canker infections?

We know that Cytospora can only enter a tree through wounds. If we knew which wounds were most important as infection sites then we might be able to reduce wounds of that type or protect these wounds with fungicides. All the cankers were observed for site of infection. We found that pruning stubs and dead twigs were the major infection sites causing 35 and 29 percent of the cankers, respectively. Winter injury cracks were the initial infection site for 14 percent of the cankers and tree crotch infections, 11 percent. There was no difference in infection sites between healthy and weak trees.

Do some varieties get more canker than others?

Our sample size was too limited to make reliable comparisons between varieties but there were some interesting trends which became evident. We found more cankers on Sunhigh than any other variety. Cresthaven had the least number of cankers. Sunhigh had about as many cankers on the trunk and secondary branches as the other varieties. There was an average of 50 cankers on primary limbs of Sunhigh compared to an average of only 4 on Cresthaven trees.

All varieties had about the same number of pruning stub infections, but Cresthaven had significantly fewer cankers arising from dead twig infections. Redhaven and Loring showed the best healing response of the varieties; 72 and 81 percent, respectively of the cankers examined were rated as very good to complete healing.

Although this information cannot be considered conclusive, it can be used to direct further research.

Is canker different in different regions of the state?

We'll consider the site of canker infection for the purpose of discussion. In Adams County, 42 percent of the cankers are a result of pruning stub infections while dead twig and winter injury account for only 21 and 18 percent of the cankers, respectively. In comparison approximately 30 percent of the cankers in the other counties were caused by pruning stubs and 30 percent by dead twigs. Again, we did not observe enough cankers to reach definite conclusions but we do have strong indication of differences between regions of the state.

Table 1. Cytospora canker infection sites.

	Percent of cankers at Infection Sites			
County	Pruning stub	Dead twig	Winter injury	Other
Adams	42	21	18	19
Franklin	30	33	10	27
York	31	30	20	19
Berks	37	36	7	20
Lehigh	33	27	14	26

EFFECT OF NEMATICIDES, TREE TRAINING AND SPACING, AND TREE QUALITY AT PLANTING ON PEACH TREE CANKER IN PENNSYLVANIA: PRELIMINARY OBSERVATIONS

B. A. Jaffee[1], J. W. Travis[2], and G. M. Greene[1]

Although bacterial canker of stone fruit has been controlled with soil fumigation in the southern and western United States, the effect of fumigants and nematicides on Cytospora canker of peach has not been investigated in the mid-Atlantic and northeastern states. In this paper, we present observations on the natural occurrence of canker in a block of peach trees (4 acres, 18 rows, planted in 1981) containing a nematicide experiment interplanted with a tree-training experiment.

In the nematicide experiment (12 rows, every third row skipped) the soil was untreated or treated with all combinations of a preplant fumigant (Telone II) and postplant nematicides (Nemacur or Furadan each spring). In the training experiment (6 rows, every third row) trees were planted in fumigated (Telone II) soil at two in-row spacings (15 or 7.5 ft) and trained to a vase or central leader system. Distance between rows was 20 feet. Trees in the nematicide experiment were obtained from nursery A (cultivar Redhaven) or nursery B (cultivar Triogem) whereas trees in the training experiment were obtained from nursery C (cultivars Cresthaven and Redskin). In the nematicide experiment, the soil was sampled three times per year for *Xiphinema americanum*, *X. rivesi*, and other plant parasitic nematodes. Nematode numbers were low for the first three years.

In 1984, all trees were rated for canker on the trunk and lower scaffolds. A high level of canker occurred in the nematicide experiment regardless of treatment or cultivar. Much less canker occurred in all treatments and cultivars of the training experiment. The difference in incidence and severity of canker in the two interplanted experiments could not be explained by nematicides, tree training, spacing, pruning, site, proximity to inoculum sources, fertilization, herbicides, or foliar pesticides. Although we cannot be certain, we believe that the severe canker in the nematicide experiment resulted from poor quality trees from the nursery; many of the trees exhibited canker and grew poorly during the first year following planting.

[1]Department of Plant Pathology and Department of Horticulture, respectively. The Pennsylvania State University, Fruit Research Laboratory, Biglerville, PA 17307.
[2]Department of Plant Pathology Extension, The Pennsylvania State University, University Park, PA 16802.

EFFICACY OF SURGICAL REMOVAL AND FUNGICIDE WOUND TREATMENTS ON ERADICATION OF CYTOSPORA CANKER ON PEACH

James W. Travis and Kenneth D. Hickey
The Pennsylvania State University
University Park, PA 16802
and
Fruit Research Laboratory
Biglerville, PA

When beginning research on a particular disease, it is not usually a good idea to begin work on eradicating the symptoms. Most people would agree that it is far better to work first on the cause of the problem and the symptoms will of course be taken care of. Even knowing this we began our canker research effort with research on the removal of the canker. There were several facotrs which influenced us in making this decision. Canker is a devastating disease in Pennsylvania peach orchards. There are not many orchards where at least some canker cannot be found. Growers felt desperate. They were literally trying anything to control the disease, and because they did not understand the disease cycle, they concentrated their efforts on the canker itself. We found that growers were applying concentrate applications of several fungicides. The method of application varied from backpack spraying of individual cankers to applying the fungicide with a paint brush. Some growers were even 'helping' the fungicide to penetrate the canker by first wire-brushing the canker. There were also some who felt that a wound paint applied after the fungicide application would improve eradication results. It goes without saying that these techniques were ineffective in eradicating the canker. There was widespread confusion as to how best to eradicate the canker, if it could be done at all. We did not try to test every procedure that was being used in the state. Instead we tested the techniques that we felt may possibly work and those that needed to be disproven. The following is a report of that work.

Procedure

The following techniques were tested; direct application of fungicides to the canker surface using a paint brush and surgical removal of the diseased tissue followed by painting on either a fungicide or water. There was also a treatment of surgery alone with no follow-up treatments.

Objective

1. To determine the effectiveness of surgically removing cankers.

2. To determine the efficacy of severe fungicide and wound treatments on canker eradication with and without surgery.

Procedure

First Year Test. Experimental blocks were located in Adams, Franklin and York counties. Treatments were applied on June 11 in Franklin County to 8 year old 'Sun Haven' peach trees; on June 12 in Adams County to 15 year old 'Early Red' peach trees and on June 16 in York County to 9 year old 'Crest Haven' peach trees. Treatments consisted of surgery or no surgery of the cankered area with follow-up treatments of Difolatan 4F, Benlate 50 W, and water or no treatment. The surgery treatment consisted of removal of diseased tissue with a knife. There were ten replications of each treatment in a randomized complete block design at each location. The treatments were applied as aqueous suspensions applied with a paint brush. Treatments were evaluated in early April. The rating system was based on the presence or absence of a complete periderm ring at the margin of the treatment area (1=healed, complete periderm, 0=not healed, incomplete periderm).

Results

The single most effective treatment was surgery (Table 1). When the diseased tissue was surgically removed, the success of canker eradication was high with or without a fungicide treatment. The surgery appeared to fail only when diseased tissue was missed during the canker removal. Likewise, when cankers were not surgically removed, canker eradication was not obtained with any of the treatments.

Table 1. The efficacy of surgery and fungicide wound treatments.

Fungicide	Percent conc. a.i.	Surgery[a]	Cultivar[b] Early Red	Sun Haven	Crest Haven
			Percent Healed[c]		
Difoltan 4F	25	+	80	100	100
	25	-	0	10	0
	2.5	+	90	100	100
	2.5	-	0	30	0
Benlate 50W	12	+	50	100	100
	12	-	0	10	0
	6	+	70	100	100
	6	-	0	30	0
water	--	+	30	90	100
	--	-	0	0	0

[a] (+) = surgery, (-) = no surgery.

[b] Treatments were applied in a randomized complete block design, ten replicates each for each cultivar. Treatments were applied in June 1981 and evaluated in April 1982.

[c] Rating system: 1 = complete periderm, 0 = incomplete periderm, total divided by the number of replicates and multiplied by 100 to equal the percent of the surgery sites healed.

Discussion

The surgery technique was very effective. The only time surgery did not eradicate the canker was when there was incomplete removal of diseased tissue.

Some individual differences were observed between treatments applied in different counties. The peach trees treated in York County (Crest Haven) were the most vigorous. Noticeably more periderm was produced at the surgery margin. Good grower management practices may have had some effect on healing; besides the obviously positive affects of fertilizer, water sprout removal from the main scaffold limbs and the mechanical topping of trees in July. This allowed better light penetration to the major scaffold limbs which may have increased vigor and periderm formation. At the Franklin County orchard the trees were of similar age and vigor but had neither sucker removal or topping, the result was the same healing success rate but a thinner periderm was formed at the surgery margin. The age and vigor of the trees in Adams County appeared to have an effect on periderm production. The trees produced noticeably less periderm at surgery margins on the major scaffold limbs than younger trees at the other two sites. Tree age and vigor appear to be a more important factor to healing after surgery than variety or post-surgery fungicide treatment.

In the same year, additional treatments were tested at a single orchard in Adams County. The treatments were: Difolatan at 25, 10, and 5% a.i., Benlate at 12% a.i., lime sulfur at 29, 14.5, and 6% a.i., Vanguard at 1% a.i., and checks (no treatment, surgery only). The results were similar to the previously reported tests; surgery was an effective method of eradication, fungicides did not improve healing after surgery, without surgery cankers did not heal, with or without fungicides.

Second Year Test. Since the most critical factors in eradicating canker appeared to be surgery and the healing response of the tree after surgery, an attempt was made to stimulate the healing process. Based on some reported success on hardwoods, a black plastic wound wrap was tried. In addition, NAA was included as a possible stimulus to periderm formation.

NAA at 50 ppm and black plastic wound wrap were applied alone and in combination with a 6% a.i. Benlate treatment immediately after surgery. Treatments were applied in a randomized complete block design. Four orchards (2 year old peach, 6 year old nectarine, 5 year old peach, and a 8 year old peach) were used as test blocks. The treatments were applied in June and evaluated the following June.

The results are contained in Table 2.

Table 2. The affect of wound dressings on healing after surgical removal of Cytospora canker.

Treatments	Test Orchards[a]			
	6 year old[1] 'LaGrande' nectarine	2 year old[1] 'Sunhigh' peach	5 year old[3] 'Marqueen' peach	8 year old[2] 'Loring' peach
	Percent Healed after Surgery[b]			
1. NAA at 50 ppm[c]	29	100	60	75
2. black plastic wound wrap	0	100	40	63
3. Benlate 50W 6% a.i. conc.	100	100	80	100
4. Benlate 50W 6% a.i. plus black plastic	100	100	60	100
5. Benlate 50W 6% a.i. plus asphalt wound dressing	100	100	100	100
6. Benlate 50W 6% a.i. white latex paint	100	100	100	100
7. Benlate 50W 6% a.i. plus NAA 50 ppm plus black plastic	57	88	100	100
8. NAA 50 ppm plus black plastic	0	100	40	75
9. no treatment, surgery only	67	100	80	100
10. no treatment, no surgery	0	88	0	57

[a] Vigor rating:
1 = vigorous (18" or more shoot growth)
2 = moderate vigor (6 to 18" shoot growth)
3 = low vigor (less than 6" shoot growth)

[b] Healing evaluation:
1 = complete periderm at surgery margin
0 = incomplete periderm at surgery margin
Total for each treatment was divided by the number of replicates and multiplied by 100 to equal percent of surgery sites healed.

[c] Treatments were applied in a randomized complete block design, ten replicates each in each orchard, in June 1982, and evaluated in June 1983.

Discussion

In brief, wound dressings did not improve the percent of healing above surgery alone in orchards which were of high to moderate vigor. However, in the nectarine block and the 'Marqueen' peach block which appeared to be more susceptible to infection and had lower vigor, respectively, there was a noticeable improvement in the percent healing of treatments which received a post surgery fungicide. The black plastic wound wrap appeared to have reduced the likelihood of healing below surgery alone. The white latex paint and asphalt paint dressings did neither improve or hinder healing after surgery. NAA at the rate applied did not stimulate the healing response after surgery.

Some interesting observations were also made which were not part of the objectives of the experiment. The two year old 'Sunhigh' peach block had been injured by a groundhog within two weeks prior to the treatments. This block was a different situation than the other blocks tested. Although wounding was substantial and subsequent infection by Cytospora was expected, many of the wounds were not yet infected. Since the trees were vigorous some wounds healed without surgery. Wound healing evidently began before infection occurred. In all of the other blocks tested only previously infected cankers were treated.

Summary

We have learned that surgically removing cankers is an effective means of control. Wound paints and black plastic wrap applied after surgery do not improve and may hinder the healing response. Fungicides may improve healing on trees or limbs of low vigor but do not improve healing on vigorous wood after surgery. Wounds made during the early part of the growing season may be healed naturally by the tree before infection occurs.

Surgical removal of canker is expensive! Or is it? Consider the cost and potential benefit. Without surgery the canker will girdle the limb or trunk and fruit production will be lost. If the canker is not removed the tree may eventually die. On the other hand, if the canker is removed by surgery, the potential for healing is good. If good management practices are followed, there may be no further need for canker removal on that tree.

In Pennsylvania, surgery is recommended in orchards three to six years old during the months of May and June. Several fungicides are recommended as post-surgery paints.

EFFECT OF PREVENTATIVE AND POST-INFECTION FUNGICIDE TREATMENTS AND THE TIME OF PRUNING ON INCIDENCE OF *CYTOSPORA* INFECTION ON PRUNING WOUNDS OF PEACH

K. D. Hickey and J. W. Travis*

Abstract

The efficacy of a single protective or post-infection fungicide spray in preventing *Cytospora* canker establishment on pruning wounds was evaluated in two commercial peach orchards in southcentral Pennsylvania. Four fungicides having different modes of action were evaluated in one experiment and four pruning dates followed by fungicide treatments appeared to have little effect on incidence of *Cytospora* infection on pruning wounds. Bitertanol, chlorothalonil, or the combination of the two provided a significant reduction in infections on wounds 0.6 to 1.3 cm in diameter when used in a single spray timed four days after an infection period. A significant increase in wound infection occurred on trees treated with copper hydroxide on small wounds but not on those 1.4 to 5.0 cm in diameter. None of the fungicide treatments were effective in preventing infections on the larger wounds. Pruning wound infections were significantly higher on trees pruned on April 11 while dormant. A sharp decline in infections occurred from April 25 to June 2, the last pruning date. A significant reduction in fruit size was observed on trees pruned on June 2. The triforine plus chlorothalonil fungicide mixture applied as a protective or post-infection spray had no measurable effect on canker establishment on pruning wounds.

Introduction

Perennial canker, caused by *Cytospora leucostoma* or *C. cincta*, is prevalent on peach and nectarine trees throughout the eastern fruit growing regions. This fungus pathogen readily invades dying or injured woody tissue caused by low winter temperature (1, 2); weakened trees from rodent injury, nematode feeding, or virus infections; or pruning wounds made during the dormant season (5). To prevent infections on pruning wounds, growers are advised (6) to delay dormant pruning until growth begins in the spring and to apply a protective fungicide treatment immediately following pruning. The efficacy of standard fungicides has been determined in limited field trials (3, 4), but very little is known of the effectiveness of fungicides registered for use in recent years or of the new sterol-inhibiting fungicides.

The efficacy of a single protective or post-infection fungicide spray in preventing *Cytospora* canker establishment on pruning wounds was determined in two commercial peach orchards in southcentral Pennsylvania (Adams County) in 1984. The treatments were applied following pruning and were superimposed on the standard commercial fungicide spray program used for brown rot. Different fungicides with different modes of action applied during the dormant season were evaluated in Experiment 1 while the effect of four pruning dates followed by a single fungicide treatment applied as a preventative or as a post-infection treatment was evaluated in Experiment 2.

*Professor and Assistant Professor of Plant Pathology, Department of Plant Pathology, The Pennsylvania State University, Biglerville and University Park, PA, respectively.

Experiment 1

Ten-year-old mature 'Marqueen' peach trees planted at 6.0 by 7.5 m in a commercial orchard were treated with five fungicide treatments applied as preventative or post-infection sprays following dormant pruning. All trees were relatively uniform with moderate growth in 1983 and had one or more established Cytospora cankers on scaffold branches or trunk. The fungicides tested were as follows: bitertanol (Baycor 50W), Mobay Chemical Co., Kansas City, MO 64120; chlorothalonil (Bravo 500F), SDS Biotech Corp., Painesville, OH 44077; copper hydroxide (Kocide 101 77W), Griffin Corp., Valdosta, GA 31603; dichlone (Quintar 540F), Hookins Agric. Chem. Co., Madison, WI 53707; triforine (Funginex 1.6 EC) FMC Corp., Philadelphia, PA 19103. The treatments were applied as a single preventative spray to pruned trees one day after pruning on April 10 before rain occurred. The post-infection treatments were applied on April 19, four days following a 27-hour rain period with a mean temperature of 10° C. Treatments were applied as dilute sprays to the point of "run-off" with a single-nozzle spray gun operated at a hydraulic pressure of 3100 kPa. Favorable environmental conditions for canker development occurred on April 22-23 during a 24-hour wetting period with a mean temperature of 6° C.

The experimental plot design was a randomized complete block with three replicates of triple-tree plots. Plots in each replicate were randomly arranged along a single row of trees with the three replicates being located in separate parallel alternate rows. The preventative and post-infection treatments were arranged in separate but adjacent rows to each other.

Experiment 2

A uniform block of six-year-old 'Sunhigh' peach trees planted 6.0 X 7.5 m in contour rows of approximately 100 trees per row were used to determine the time of pruning wound infection and the effectiveness of a fungicide spray in preventing canker establishment. The trees were in moderate to high vigor and less than 20 percent had established Cytospora canker on scaffold branches or trunk. One complete row of trees was pruned on Apr. 11, Apr. 25, May 9, and June 2 which corresponded approximately with the dormant, pink, late-bloom, and two weeks following shuck-fall phenological growth stages, respectively. Following each pruning date, a single spray application of a mixture of Funginex 1.6 EC 180 mg ai/L (12 fl. oz./100 gal.) plus Bravo 500F 1663 mg ai/L (43 fl. oz./100 gal.) was applied to a set of plots as a protective fungicide before infection occurred. A second series of plots was sprayed with the same mixture following a wetting period which was favorable for pruning wound infection. No fungicide was applied to these plots before rain occurred. The intervals between the four pruning dates and the post-infection spray were 7, 6, 20, and 10 days, respectively. A third set of plots in each row was not sprayed with the fungicide mixture and served as untreated checks.

The experimental plot design was a randomized complete block with three replicates of 10-12 trees per plot arranged along each of the pruned rows. Adjacent rows of trees were pruned on each of the pruning dates. The fungicide treatment was applied as in Experiment 1 and was superimposed on the standard commercial fungicide program con-

sisting of a combination of benomyl plus captan in the pink and bloom periods and a mixture of captan plus sulfur during the post-bloom period.

Data acquisition and analyses. In determining incidence of infection on pruning wounds based on numerous isolations in previous tests (2, 3, 5), infection was presumed to be positive if gum formation was apparent around the cambial zone. Wounds showing no gum formation was presumed to be free of infection.

In each of the experiments, pruning wounds ranging from 0.6 to 1.3 cm and those ranging from 1.4 to 5.0 cm in diameter were examined and recorded separately. In Experiment 1, ten wounds of each size were observed on the center tree of each three-tree plot. In Experiment 2, ten wounds of each size were observed on each of three trees per ten-tree plot. All data sets collected were statistically analyzed using standard analysis of variance for randomized block design and the Duncan's Multiple Range Test for mean separation.

Results

Frequent rain periods with temperature ranging from 1.0° to 15.0° C were highly favorable for infection during the first half of April. Cytospora incidence on pruning wounds 0.6-1.3 cm in diameter in the 'Marqueen' orchard (Experiment 1) ranged from 20-53 percent on the untreated checks. Incidence was 53-73 percent on wounds 1.4-5.0 cm in diameter on untreated trees in the protective and post-infection tests, respectively. Baycor, Bravo, and the combination of the two provided some disease control but not significant from the untreated when used as protectants. The combination also was more effective when used in the post-infection test. None of the treatments were highly effective in preventing infection on wounds 1.3-5.0 cm in diameter. There was no significant difference between protective or post-infection treatments.

In Experiment 2, there was significantly more infection on pruning wounds made on Apr. 11, but no significant difference among other dates. Pruning wounds 1.4 to 5.0 cm in diameter had significantly higher infection rates than those smaller than 1.4 cm. The fungicide treatment had little effect in preventing infection of pruning cuts when used immediately following pruning or after infection had occurred.

Literature cited

1. Hampson, M. C. and W. A. Sinclair. 1973. Xylem dysfunction in peach caused by Cytospora leucostoma. Phytopathology 63: 676-681

2. Helton, A. W. and H. Randall. 1975. Cambial gummosis in Prunus domestica infected with Cytospora cincta. Plant Dis. Rep. 59: 340-344.

3. Hickey, K. D. and K. G. Parker. 1963. Control of perennial canker of peach. Amer. Phytopathological Soc. Fungicide and Nematicide Test Results 19: 50-51.

4. _____ 1964. Control of perennial canker of peach. Amer. Phytopathological Soc. Fungicide and Nematicide Test Results 20: 45-46.

5. Jones, A. C. and N. S. Luepschen. 1971. Seasonal development of Cytospora canker on peach in Colorado. Plant Dis. Rep. 55(4): 314-317.

6. Travis, J. W. and et al. 1984. Tree Fruit Production Guide for Pennsylvania. The Pennsylvania State University, Cooperative Extension Service. 80 pp.

Table 1. Percent Infection of Pruning Wounds on 'Marqueen' Peach Trees Treated with Fungicides Applied as Dilute Protectant or Post-Infection Sprays in 1984. Fairview Farm, Gettysburg, PA

Fungicide and amt. mg ai/L (Form./100 gal.)	Mean Percent Infection* Pruning Wound Size			
	0.6-1.3 cm		1.3-5.0 cm	
	Prot.[1]	PI[2]	Prot.[1]	PI[2]
Baycor 50W 150 (4.0 oz)	0.0b	33.3ab	53.3ab	90.0a
Bravo 500F 1663 (43 fl oz)	13.3b	16.7bc	36.7b	56.7b
Bravo 500F 1663 (43 fl oz) + Baycor 50W 150 (4.0 oz)	6.7b	6.7c	86.7a	60.0b
Quintar 540F 625 (16 fl oz)	33.3b	16.7bc	73.3ab	80.0ab
Kocide 101 77W 2768 (3.0 lb)	83.3a	30.0bc	86.7a	86.7a
Untreated Check	20.0b	53.3a	53.3a	73.3ab

[1]Fungicide treatments were applied dilute to point of run-off (2340 L/ha) one day following pruning on April 10 before rain occurred.

[2]Fungicide treatments were applied on April 19, eight days after trees were pruned and four days following a wetting period favorable for infection.

*Means followed by the same letter(s) are not significantly different (P=0.05, DMRT).

Table 2. Percent Infection of Pruning Wounds on 'Sunhigh' Peach Trees Treated with a Protectant or Post-Infection Fungicide Spray on Four Pruning Dates in 1984. Lerew Orchards, York Springs, PA

Pruning wound 0.6-1.3 cm in diameter

	Mean Percent of Pruning Wounds Infected* Pruning Dates			
Fungicide Type[1]	4/11	4/25	5/9	6/2
Protectant	12.2a	1.1a	3.3a	0.0a
Post-Infection	13.3a	5.6a	2.2a	2.2a
Untreated Check	10.0a	3.3a	2.2a	2.2a
Pruning wound 1.3-5.0 cm in diameter				
Protectant	36.7a	7.8a	4.4b	3.3a
Post-Infection	21.1ab	14.4a	6.7b	4.4a
Untreated Check	17.0b	10.0a	14.4a	3.3a

[1]A combination of Funginex 1.6EC 180 mg ai/L (12 fl. oz./100 gal.) plus Bravo 500F 1663 mg ai/L (43 fl. oz./100 gal.) was applied dilute in a single application following pruning before rain occurred (protectant) or following a wetting period favorable for infection (post-infection)

*Means followed by the same letter(s) are not significantly different (P=0.05, DMRT).

SOIL MANAGEMENT AND IRRIGATION EFFECTS ON PEACH CANKER IN THE NIAGARA PENINSULA OF ONTARIO

R.A. Cline, Research Scientist
Horticultural Research Institute of Ontario
Vineland Station, Ontario L0R 2E0

Abstract

An experiment to study the effects of systems of soil management on performance of Redhaven and Loring peach was concluded in 1981. Growth and yield of Redhaven were larger with the sod and mulch system of soil management than with the more conventional cultivation and cover crop system. Loring yields were the same with both systems of soil management. Winter injury and peach canker ratings were less for both cultivars under the sod conditions than with cultivation.

In another experiment, peach canker has been less when trees were trickle irrigated than when not. Irrigating until mid-September had a greater effect than when irrigation was stopped in early August.

Introduction

Soil management can have an important role in peach canker and tree longevity. A number of soil management factors should be considered to minimize the effects of cold temperature injury and peach canker. Willison (2) showed the effects of length of cultivation on canker development. The number of cankers increased progressively and dramatically when cultivated and continued until July 15 to August 15 compared to stopping June 15. In Ontario, most orchards are cultivated and it is recommended that cultivation be stopped by July 1. It is good practice to seed a cover crop at this time to help slow up growth and harden the trees in preparation for winter. This also helps maintain soil organic matter.

Fertilizers can also play an important part in peach canker. Cline (1) reported, an increase, as expected, in canker at high levels of nitrogen. Low levels of nitrogen were also found to lead to increased canker. Maintaining a balance between N and K is extremely important. Higher levels of N may be tolerated if N is kept in balance with K. Excessive N should be avoided by applying only moderate rates of N fertilizer and by applying it early. We have found no differences in peach canker and dead wood between the same rate of N applied as ammonium nitrate or calcium nitrate (Table 1).

Table 1. THE EFFECT OF SOURCE OF NITROGEN ON CANKER RATING AND % DEADWOOD IN PEACH AT VINELAND IN 1982

	Canker Rating*	% Deadwood
$Ca(NO_3)_2$	5.4	17.4
NH_4NO_3	4.9	17.3

*Canker rated from 0 (none) to 10 (severe).

In 1972 an experiment was initiated to study the effects of soil management factors in the growth, yield and severity of peach canker. A randomized block design was used with the following soil management methods as main blocks:

1. Cultivation until July 1 when ryegrass was seeded.

2. Creeping red fescue seeded as a permanent sod in the third growing season (July 1974). A mulch of hay was maintained under the spread of tree branches beginning in the fall of 1974.

Sub-blocks included two rates of nitrogen and two rates of potassium shown in Table 2. N fertilizer was applied in early April each year while K was applied in May.

Table 2. FERTILIZER APPLIED IN ALL COMBINATIONS

N_1 - 19 g N/yr. x age of tree
N_2 - 38 g N/yr. x age of tree
K_1 - based on leaf analyses (up to 400 g K_2O/tree)
K_2 - 2 X K_1

Two varieties (Redhaven, Loring) were grafted to two rootstocks (Elberta, Bailey) which were planted in each plot. Tree spacing was 3.1m in the row with 6.2m between rows. In 1979 every other tree was removed in the row leaving one tree of each variety rootstock combination in each plot at a spacing of 6.2 x 6.2 m.

Data were collected for yield, growth (change in cross sectional area). Leaf composition was determined for July and September samples. The extent of canker and deadwood was rated each spring.

Redhaven yielded more under sod conditions than under cultivation except with the N_2K_1 treatment (Table 3). Loring on the other hand tended to yield more with cultivation but differences were small. Yields of Redhaven were increased by increased N rates with cultivated conditions but responded more to K under sod conditions. In fact, at the low rate of K under sod conditions, increased N tended to decrease Redhaven yields.

Table 3. THE EFFECT OF SOIL MANAGEMENT AND FERTILIZER ON CUMULATIVE YIELD LB/TREE OF REDHAVEN AND LORING 1974-82

	Redhaven		Loring	
	Sod	Cult.	Sod	Cult.
N_1K_1	1541	1186	1190	1256
N_2K_1	1410	1434	1288	1275
N_1K_2	1558	1230	1296	1349
N_2K_2	1621	1425	1223	1318

At the end of the experiment more trees of both cultivars were alive with sod conditions than with cultivated conditions (Table 4) at all fertilizer rates. Increased N tended to reduce Loring tree survival at both levels of K and at the low level of K for Redhaven under sod conditions.

Table 4. THE EFFECT OF SOIL MANAGEMENT AND FERTILIZER ON TREE SURVIVAL AT THE END OF THE EXPERIMENT

	Trees Alive %			
	Redhaven		Loring	
	Sod	Cult.	Sod	Cult.
N_1K_1	100	38	75	38
N_2K_1	88	50	50	12
N_1K_2	100	25	75	25
N_2K_2	100	25	50	25

The extent of canker was rated from 0 (none) to 10 (extreme) several times including June 15, 1982, as shown in Table 5.

Table 5. THE EFFECT OF FERTILIZER AND SOIL MANAGEMENT TREATMENTS ON CANKER RATING* - JUNE 15/82

	Redhaven		Loring	
	Sod	Cult.	Sod	Cult.
N_1K_1	3.4	6.7	7.4	7.9
N_2K_1	3.8	6.3	8.1	9.4
N_1K_2	3.5	8.4	7.4	7.9
N_2K_2	3.5	8.4	7.2	8.7

* Canker rated from 0 (none) to 10 (extreme).

At all levels of fertilizer canker was less for both cultivars under sod conditions than under cultivated conditions (Table 5). Canker was more severe with Loring which is more sensitive to cold temperature injury. However, the effect of sod in reducing canker rating was larger with Redhaven.

Fertilizer rate had little effect on canker rating under sod conditions except at the low rate of K when increased N resulted in more canker. At the high rate of K this was not true. Under cultivated conditions canker rating of Loring was increased at both levels of K by increased N, but the effect was less at the high rate of K. In contrast, Redhaven tended to have more canker at the high rate of K than at the low rate at both levels of N.

In summary, the sod system of soil management resulted in less canker and better tree survival for both Redhaven and Loring. The sod system of soil management is becoming more popular with Ontario growers.

In another experiment, the effects of trickle irrigation on the growth, yield and canker development of three peach cultivars was studied. Earlired (early), Redhaven (mid-season) and V59091 (late) were selected to give a range in maturity. Trees were planted the spring of 1973 on a Vineland fine, sandy loam which has a high moisture holding capacity. Tree spacing was 3.1 x 6.2m. Irrigation was initiated the year of planting commencing in early July and continuing until August 1 or mid-September. Microtube emitters delivered 4.5L/hr. Initially one emitter was placed at each tree. Later an additional emitter was placed between trees. The amount of water appled was adjusted to maintain tensiometer readings at the 30cm depth below 30 centibars.

Fertilizer treatments shown in Table 6 were applied with the irrigation water. All treatments were compared to a non-irrigated check. Irrigation increased tree survival in the very dry years of 1973 and 1974 after planting. The cumulative yield of the late variety of V59091 was increased the most by irrigation. Only data for this variety is shown. Irrigation all season was most effective in increasing yields. Fertilizer with the irrigation water decreased yields compared to water alone. KCl late in the season resulted in higher yields than KNO_3 late.

Table 6. THE EFFECT OF IRRIGATION AND FERTILIZER ON CUMULATIVE YIELD 75-83 OF V59091

	Irrigated Until Sept. 15	Irrigated Until Aug. 1
	lb/tree	
KNO_3 Throughout	623	432
NH_4NO_3 early KCl late	716	486
KNO_3 early KCl late	767	---
Water only	804	710
Not Irrigated	649	613

Canker of V59091 was less in May 1982 when trees were irrigated than when not (Table 7). Canker tended to be greater at that time when irrigation was continued until mid-September compared to stopping irrigation August 1. Early in this study (1) irrigation until September 15 resulted in less canker than when irrigation was stopped August 1. Fertilizer with irrigation water did not result in lower canker ratings in May 1982. However, when KCl was used late in the season rather than KNO_3, canker was less. This confirms earlier findings (1) which indicate less canker when K fertilizer was applied late in the season.

Table 7. THE EFFECT OF IRRIGATION AND FERTILIZER TREATMENTS ON CANKER RATINGS* OF V59091, MAY 1982

Fertilizer	Irrigated Until Sept. 15	Irrigated Until Aug. 1
KNO_3 throughout	6.8	6.0
NH_4NO_3 early KCL late	6.6	5.8
KNO_3 early KCl late	5.4	4.5
Water only	5.4	5.0
Not Irrigated	6.2	5.9

*Canker rated from 0 (none) to 10 (severe).

In conclusion, over the life of this experiment canker was less with irrigation than without. KCl fertilizer in the irrigation water late in the season resulted in less canker.

Literature Cited

1. Cline, R.A. (1980). Orchard Management and Peach Canker. Peach Canker Workshop Procedings compiled and edited by R.E.C. Layne, Research Station, Agriculture Canada, Harrow.

2. Willison, R.S. (1933). Peach canker investigations and some notes on incidence, contributing factors and control measures, Sci. Agric. 14:32-47.

PATHOGENIC AND COMPETETIVE CAPABILITIES OF *CYTOSPORA CINCTA*

Elke Endert
Department of Plant Pathology
North Carolina State University
Raleigh, N.C. 27695-7616

Cytospora spp. have traditionally been regarded as secondary invaders of stressed or injured trees. Many hardwood and conifer species are susceptible to at least one *Cytospora* sp., especially following drought or extremes in temperature. *C. cincta* and *C. leucostoma* are known to induce cankers in stone fruit. Although both species infect peach trees, *C. cincta* is more predominant and expresses far more virulence in peach than does *C. leucostoma* (20). Cytospora canker of peach is often termed "perennial canker" because infections cease to develop in the fall and resume activity in the following growing season. In the Southeast, however, peach trees which are injured by sudden late-winter freezes are often rapidly colonized by *Cytospora* spp. within a few weeks (17). Despite numerous attempts to define the conditions favoring such extensive invasions, these conditions remain unknown.

Cytospora canker and bacterial canker (caused by *Pseudomonas syringae* pv. *syringae*, hereafter 'Pss') are considered the most common biotic factors directly associated with tree death in peach tree short life (PTSL) (17). In recent experiments aimed at clarifying the respective roles of bacterial and Cytospora canker, several phenomena were diagnosed concerning *C. cincta* and its interactions with Pss. The most pertinent data were obtained from co-inoculation of both pathogens followed by re-isolation. The objective of this report is to (1) describe preliminary results of 1983 and 1984 experiments and (2) discuss their implications on the role of *C. cincta* within the PTSL complex.

Variation in Virulence Among Strains

Strains of *C. cincta* and *C. leucostoma* have been shown to vary in virulence, host specificity, and reduction of substrate pH (10,22). Differences in canker incidence among peach orchards have been attributed to variations in levels of virulence (22). Two or more strains exhibiting different cultural morphologies can often be isolated within one tree or even one canker (C. N. Clayton, personal communication). These reports suggest that if certain morphological or physiological characteristics were consistently associated with high levels of virulence, such characteristics could be utilized for rapid screening of large numbers of isolates. Experiments were therefore initiated to measure various characteristics of *C.*

cincta strains with the long-term objective of determining fitness parameters. Although most of these studies are preliminary, the following two progress reports contribute some insight into the variation in virulence among strains.

Variation in virulence among two morphologically distinct strains. Two strains of C. cincta originally isolated from peach were selected for their differing growth patterns on malt agar: strain 8.2 - rapid growth, entire margin; and strain 10.2 - slower growth, deeply lobed margin. These strains were inoculated into scaffold limbs of 7-yr-old 'Redhaven' trees, using hammer wounds (7), in November 1983 and January and March 1984. Canker measurements taken in April 1984 showed that strain 10.2 induced larger cankers than 8.2 when inoculated in autum (Table 1) (P=0.05). Inoculations made in winter or spring resulted in either small cankers or callus formation, so that strain differences were not significant for those inoculations. Measurements of inner bark pH were also taken using a flat-surface electrode. Strain 10.2 reduced the bark pH significantly lower than did strain 8.2 (Table 1) (P=0.05). These data suggest that reduction in bark pH may contribute to the variation in virulence expressed among strains of C. cincta. Experiments are currently being conducted to determine whether this relationship is consistent in vitro.

Expression of virulence in 1-yr-old trees. Forty-two strains of C. cincta isolated from peach in 1983 were inoculated onto 1-yr-old 'Na-2' and 'Na-8' peach seedlings in November 1983. Five-mm mycelial plugs were placed onto 2 cm pruning stubs and wrapped with Parafilm to avoid dessiccation. Extent of canker development was evaluated in May 1984 according to a 0 to 4 rating scale:

0 = no bark infection; 1 = infection of pruning stub; 2 = stem canker; 3 = girdling of distal limbs; 4 = girdling of proximal limbs. Thirty-eight of the 42 strains induced disease ratings of 2 or greater; two of these strains consistently induced canker lengths greater than 8 cm. The remaining four strains did not induce externally visible symptoms; however, the outer wood within pruning stubs was discolored in comparison to controls.

To determine the extent of internal colonization, 32 trees were divided into five segments, surface-disinfested, and separated into bark, wood, and pith components. Each component was duplicate-plated onto malt agar and evaluated for isolation of C. cincta. The results (Fig. 1) indicate that the fungus was reisolated from all three components along the entire main stem of the tree. Reisolations were consistently more frequent from wood than from either bark or pith (Fig. 1). This data suggests that C. cincta is capable of invading the wood of peach trees. Such deep- seated infections may explain the rapid colonization of freeze-injured trees when no previous cankers were evident.

Competition Between C. cincta and PSS

Simultaneous inoculation of C. cincta and Pss into apricot bark in Hungary resulted in larger cankers than occurred with either pathogen alone (18). The authors commented, however, that Pss was rarely reisolated (18). Similar difficulties have been reported in the Southeast (3, 17), especially with naturally-occurring infections. Of 120 trees showing freeze-injury or bud necrosis in 1983, C. cincta and Pss were isolated at frequencies of 76 and 5%, respectively (D. F. Ritchie, preceding manuscript). Similar ratios occurred in 1982. The following two studies were conducted to (1) determine the relative frequencies at which C. cincta and Pss occur in injured peach tissues and (2) monitor the survival of both pathogens following simultaneous inoculation into peach trees.

Frequency of isolation. Between February and May 1984, 226 samples representing 198 peach trees in the N.C. Sandhills region were surface-disinfested and plated in duplicate onto malt agar and King's medium B. Identification of fungal cultures as C. cincta was based on pycnidial morphology and pigmentation (20). Fluorescent bacteria were identified as Pss using biochemical tests (3) and were tested for hypersentitive reaction in tobacco and lesion induction in peach leaves (3). Various yeasts were also isolated, usually in association with either C. cincta or Pss. Of the fluorescent bacteria isolated, many were not identified as Pss, and less than 50% were pathogenic (Table 3). No organism was consistently associated with any specific type of symptom. However, the ratio of pathogenic Pss to total fluorescent bacteria was larger in twigs and buds than in trunks or scaffold limbs (Table 3).

Competition between C. cincta and Pss. C. cincta strain 4A and Pss strain B-15+ rif 2 were inoculated simultaneously or separately into scaffold limbs of 7-yr-old 'Loring' trees on 'Lovell' rootstock in November 1983 using hammer wounds (7). Fungal inoculum consisted of 5 mm mycelial plugs from 2 da malt agar cultures; bacterial suspensions of 36 hr cultures were applied at 10^7 cfu/ml onto sterile 2x2 cm patches of gauze. Controls

consisted of sterile malt agar or sterile distilled water and gauze, respectively. Inoculations were repeated in March 1984. All results were evaluated in April 1984.

Inoculation of Pss in fall resulted in larger cankers than inoculations of both pathogens or C. cincta alone (Fig. 2A). Analysis of reisolations, however, indicated that 11 out of 18 Pss inoculation sites were colonized by C. cincta, whereas Pss was no longer detected. Pss was also not reisolated from co-inoculated sites. Fourteen and 17 C. cincta were isolated from single and co-inoculated sites, respectively. Five of 18 control wounds were colonized by C. cincta.

When Pss was inoculated in the spring, 15 of 18 sites contained detectable Pss at time of sampling, whereas no viable Pss were detected in co-inoculated sites. C. cincta was reisolated from all single and co-inoculation sites. Cankers resulting from C. cincta or C. cincta plus Pss were similar (Fig. 2A). C. cincta was reisolated from two and five of the control and Pss sites, respectively.

The pH of inner bark 2-5 cm from the inoculation site was determined immediately after canker measurements were taken. Flat incisions were made parallel to the cambium both distal and proximal to the inoculation site and hydrated with 100 ul sterile deionized water. A flat-surface electrode was used to record pH (+ 0.05). Distal and proximal readings were averaged for each inoculation site. Fall inoculation of C. cincta reduced the bark pH to 4.1, compared to pH 5.0 in control wounds (Fig. 2B). Spring inoculation also resulted in reduced bark pH, though to a lesser degree (Fig. 2B). The average pH of bark in scaffold limbs of non-wounded trees was 5.20 (std. error + 0.14, sample size 17 limbs).

These data appear similar to the "synergistic" effect reported in Hungary (18). Wounds which contained both Pss and C. cincta seemed to produce larger cankers than wounds containing C. cincta alone (Fig. 2A). However, Pss did not survive in detectable numbers, suggesting that its continued contribution to canker development was unlikely. Repeated exposures to various hydrogen-ion concentrations have ascertained that Pss cannot survive at pH below 4.6 (D. F. Ritchie, preceding manuscript). This sensitivity to low pH may explain why Rozsnyay and Klement (18) experienced difficulty in reisolating Pss from co-inoculations. Pss by itself did not induce measurable cankers (Fig. 2A); those cankers which formed were confirmed to be colonized by naturally-occurring C. cincta strains. Why C. cincta preferred to invade Pss-inoculated wounds over controls is still unclear.

Production of Oxalic Acid and Pectinolytic Enzymes

Several Cytospora spp. reduce the pH of their substrate during growth (4,10). This characteristic is common to many cell wall-degrading plant pathogens which excrete oxalic acid (6,14,15). Observation of C. cincta grown on malt agar revealed the presence of numerous prismatic crystals which were insoluble in water ands resembled calcium oxalate (unpublished). Presence of oxalic acid in liquid cultures was confirmed by titration against known standards (6,15).

Excretion of oxalic acid by plant pathogens serves to lower the extracellular pH to the optimum required by pectinolytic enzymes. Removal of calcium from plant cell walls (to precipitate calcium oxalate) furthermore increases the susceptibility of cell walls to pectinolytic enzymes (6,14,15). Infection of poplar by C. chrysosperma appears to involve the degradation of pectins (2) and similar evidence has been suggested for C. cincta (1). Studies were therefore initiated to clarify the pathogenic capabilities of C. cincta strains isolated from peach.

Oxalic acid. Five strains of C. cincta were selected to represent low, moderate, and high levels of virulence. Seven 5 mm plugs of malt agar cultures were transferred to 50 ml malt broth containing 0.2% citrus pectin and incubated for 6 days in shaking culture at room temperature (20-22C). Cultures were filtered through Whatman No. 4 paper. The cell-free culture medium was partially purified and titrated against potassium permanganate to determine the concentrations of oxalic acid (6,14,15). No differences in oxalic acid levels were detected among these strains.

To determine the effect of pectin on production of oxalic acid, strain 9.2 was incubated for 6 da in 25% potato dextrose broth amended with 1% w/v pectin and other organic compounds. Levels of oxalic acid were similar with all compounds (Table 2). Final pH varied greatly, suggesting that the reduction in substrate pH cannot be attributed to oxalic acid alone. Polygalacturonic acid (PGA) and carboxy-methyl cellulose (CMC), which enhance oxalic acid production in Sclerotium rolfsii (15), did not affect oxalic acid production in C. cincta (Table 2).

Pectinolytic enzymes. Cell-free culture extracts of strain 9.2 were incubated with carrot discs to determine the presence of pectin-degrading enzymes (14). Tissue maceration was rated after 24 hr on a 0 to 3 scale, 3 representing total maceration. Presence of pectinolytic compounds was detected only when pectic compounds were included in the incubation medium (Table 2). Incubation of strain 9.2 in 0.2% citrus pectin demonstrated that excretion of pectinolytic enzymes was temporally preceded by reduction in substrate pH and production of oxalic acid (Fig. 3).

Utilization of cellulose. Histological observation of Cytospora canker of poplar has suggested that C. chrysosperma may be capable of degrading cellulose (2). Presence of cellulolytic enzymes can be diagnosed in a synthetic medium containing cellulose which develops a clearing zone upon degradation of cellulose (16). No degradation of cellulose was detected in C. cincta strains isolated from peach.

Growth on carbon sources. C. cincta strains 9.2 and 10.4 were incubated on a synthetic medium (11) using potassium nitrate as the nitrogen source (8). Twelve compounds were selected as sole carbon sources (Fig. 4). The pH of each medium was adjusted to 6.0 before autoclaving (9). Three growth responses were measured: radial growth (Fig. 4A), time required for the initial appearance of pigment (Fig. 4B), and an artificial sporulation rating based on a 0 to 5 scale (Fig. 4C). Some growth occurred on the carbonless medium due to reserve nutrients within the transfer plug; therefore growth on all carbon sources was evaluated in relation to the control. Growth of both strains was best on citrus pectin (Fig. 4A,B,C). Tannic acid served as the second-best carbon source. In general, pectic substances served as better carbon sources than sugars (Fig. 4).

Conclusions and Future Directions

The ability of C. cincta to inhibit Pss, to excrete enzymes capable of macerating plant tissue, and to utilize pectin and tannin support the observation that C. cincta is an extremely ubiquitous and important pathogen of peach trees in North Carolina. The ability to grow on tannic acid suggests that it is unlikely for C. cincta to assume a secondary role in bark invasion, since tannic acid is inhibitory to secondary pathogens (19). In addition, the consistent colonization of wood provides a plausible explanation for the rapid infection of peach trees that is characteristic of PTSL. Other Cytospora spp. have been implicated in blue streaking of softwood timbers and C. cincta may possess similar capabilities.

Current strategies to reduce PTSL by maintaining tree vigor still appear most feasible. Inoculum of Cytospora spp. may be continuously available throughout the year (12) and has been difficult to eradicate or reduce through fungicidal spray programs. However, the pathogenic strategy of C. cincta may suggest an alternative method for disease control. Application of calcium nitrate has recently proven effective for control of soft rot of potato (13). Similar control may be achieved in similar diseases in which the amount of oxalic acid produced by the pathogen is limited. Such newer approaches may prove adequate in reducing or perhaps preventing infection of stressed or injured peach trees.

LITERATURE CITED

1. Banko, T.J., & Helton, A.W. 1974. Cytospora-induced changes in stems of Prunus persica. Phytopathology 64: 899-901.

2. Biggs, A.R., Davis, D.D., & Merrill, W. 1982. Histo- chemical aspects of Cytospora canker of hybrid poplar. Phytopathology 72: 705 (abstr.).

3. Endert, E. 1982. Movement and overwintering of Pseudomonas syringae pv. syringae in peach twigs. Proc. Stone Fruit Decline Workshop, Michigan State Univ., Oct. 18-20, 1982, pp. 103-112.

4. Endert-Kirkpatrick, E., & Ritchie, D.F. 1984. Competition among bark parasites associated with peach tree short life. Phytopathology 74: 627 (abstr.).

5. English, H., Lownsbery, B.F., Schick, F.J., & Burlando, T. 1982. Effect of ring and pin nematode on the development of bacterial canker and Cytospora canker in young French prune trees. Plant Dis. 66: 114-116.

6. Havir, E.A., & Anagnostakis, S.L. 1983. Oxalate production by virulent but not by hypovirulent strains of Endothia parasitica. Physiol. Plant Pathol. 23: 369-376.

7. Helton, A.W. 1962. Relative efficiency of three methods of inoculating tree stems with Cytospora fungi. Phytopathology 52: 1226-1228.

8. Helton, A.W., & Konicek, D.E. 1962. An optimum environment for the culturing of Cytospora isolates from stone fruits. III. Nitrogen sources. Mycopath. Mycol. Appl. 16: 125-132.

9. Konicek, D.E., & Helton, A.W. 1962. An optimum environment for the culturing of Cytospora isolates from stone fruits. II. Carbon sources. Mycopath. Mycol. Appl. 16: 27-34.

10. Konicek, D.E., & Helton, A.W. 1962. An optimum environment for the culturing of Cytospora isolates from stone fruits. IV. Hydrogen-ion concentration. Mycopath. Mycol. Appl. 16: 243-248.

11. Lilly, V.G., & Barnett, H.L. 1951. Physiology of the fungi. McGraw-Hill Co., Inc., New York, 464 pp.

12. Luepschen, N.S., & Rohrbach, K.G. 1969. Cytospora canker of peach trees: spore availability and wound susceptibility. Plant Dis. Reptr. 53: 869-872.

13. McGuire, R.G., & Kelman, A. 1984. Reduced severity of Erwinia soft rot in potato tubers with increased calcium content. Phytopathology 74: 1250-1256.

14. Punja, Z.K., Huang, J.-S., & Jenkins, S.F. 1984. The relationship of growth and production of oxalic acid and cell wall

degrading enzymes to virulence in Sclerotium rolfsii. Can J. Plant Pathol. 6: (in press).

15. Punja, Z.K., & Jenkins, S.F. 1984. Influence of medium composition on mycelial growth and oxalic acid production in Sclerotium rolfsii. Mycologia 76: 547- 550.

16. Rautela, G.S., & Cowling, E.B. 1966. Simple cultural test for relative cellulolytic activity of fungi. Appl. Micro. 14:892-898.

17. Ritchie, D.F., & Clayton, C.N. 1981. Peach tree short life: a complex of interacting factors. Plant Dis. 65: 462-469.

18. Rozsnyay, D.S., & Klement, Z. 1973. Apoplexy of apricots. II. Cytosporal die-back and the simultaneous infection of Pseudomonas syringae and Cytospora cincta on apricots. Acta. Phytopathol. Sci. Acad. Hung. 8: 57-69.

19. Russin, J.S., & Shain, L. 1984. Colonization of chestnut blight cankers by Ceratocystis microspora and C. eucastanae. Phytopathology 74: 1257-1261.

20. Willison, R.S. 1936. Peach canker investigations. II. Infection studies. Can. J. Res. 14: 27-44.

21. Wutcher, H.K., Del Valle, N., & de Bernard, A. 1983. Citrus blight and wood pH in Cuba and Florida. HortScience 18: 486-488.

22. Wysong, D.S., & Dickens, L.E. 1962. Variation in virulence of Valsa leucostoma. Plant Dis. Reptr. 46: 274-276.

Table 1. Results of hammer-wound inoculations of two Cytospora cincta strains into scaffold limbs of seven-yr-old 'Redhaven' peach trees.[a]

Strain	Date of Inoculation: 11 Nov 1983 — Canker length[b]	11 Nov 1983 — Bark pH[c]	17 Jan 1984 — Canker length	17 Jan 1984 — Bark pH	22 Mar 1984 — Canker length	22 Mar 1984 — Bark pH
8.2	4.4	4.46	2.4	4.94	4.3	4.21
10.2	12.9	4.04	2.0	4.93	5.0	4.15

a/ Results were evaluated 26 Apr 1984; means of 9 observations per treatment

b/ measured as cm of necrotic bark

c/ pH of inner bark as recorded using a flat-surface electrode

Table 2. Results of incubation of Cytospora cincta strain 9.2 in 25% potato dextrose broth with various amendments.[a]

Amendment	Mycelial dry wt (mg)[b]	Final pH[c]	Oxalic acid (mg)	Maceration rating[d]
none	87.4	3.04	0.9	0.0
dextrose	135.9	2.95	1.8	0.0
maltose	131.0	3.04	1.3	0.0
citrus pectin	156.1	3.01	1.3	2.9
Na-polypectate	227.5	3.72	1.4	2.9
polygalac-turonic acid	55.6	4.31	1.4	2.6
α-cellulose	-[e]	3.06	1.2	0.0
carboxymethyl-cellulose	97.5	5.02	1.2	0.0

a/ Measurements were taken after six da incubation; means of eight replications.

b/ compounds were added at concentrations of 1% (w/v)

c/ pH was adjusted to 7.0 before autoclaving

d/ degree of maceration of three carrot discs, measured on a 0-3 rating scale, 0 representing unmacerated tissue

e/ not measurable due to insoluble sediments

Table 3. Results of reisolations from 226 samples from 198 peach trees in North Carolina between February and May 1984.

Symptom	Total # samples	Cytospora fungi						Yeasts		Fluorescent Pseudomonads					
		Fruiting bodies		Cytospora spp.		C. cincta				Fluorescent		Similar to Pss[a]		Pathogenic Pss	
		# samples	%	# samples	%	# samples	%	# samples	%	# samples	%	# samples	%	# samples	%
TRUNK & SCAFFOLD LIMBS															
cambial injury	173	1	0.6	52	30.1	52	30.1	73	42.2	44	25.4	26	15.0	15	8.7
bark necrosis	134	3	2.2	46	34.3	44	32.8	57	42.5	30	22.4	20	14.9	13	9.7
sour sap	112	1	0.9	39	34.8	39	34.8	47	42.0	25	22.3	16	14.3	10	8.9
limited canker	32	0	0.0	9	28.1	9	28.1	6	18.8	9	28.1	8	25.0	4	12.5
callus near injured cambium	7	0	0.0	4	57.1	4	57.1	2	28.6	2	28.6	0	0.0	0	0.0
TWIGS & BUDS															
browning of xylem	37	2	5.4	20	54.1	20	54.1	32	86.5	10	27.0	5	13.5	3	8.1
bud necrosis	32	2	6.2	22	68.8	22	68.8	28	87.5	7	21.9	4	12.5	3	9.4
water-soaking	10	1	10.0	6	60.0	6	60.0	9	90.0	5	50.0	3	30.0	2	20.0

a/ similar in biochemical characteristics to *Pseudomonas syringae* pv. *syringae*

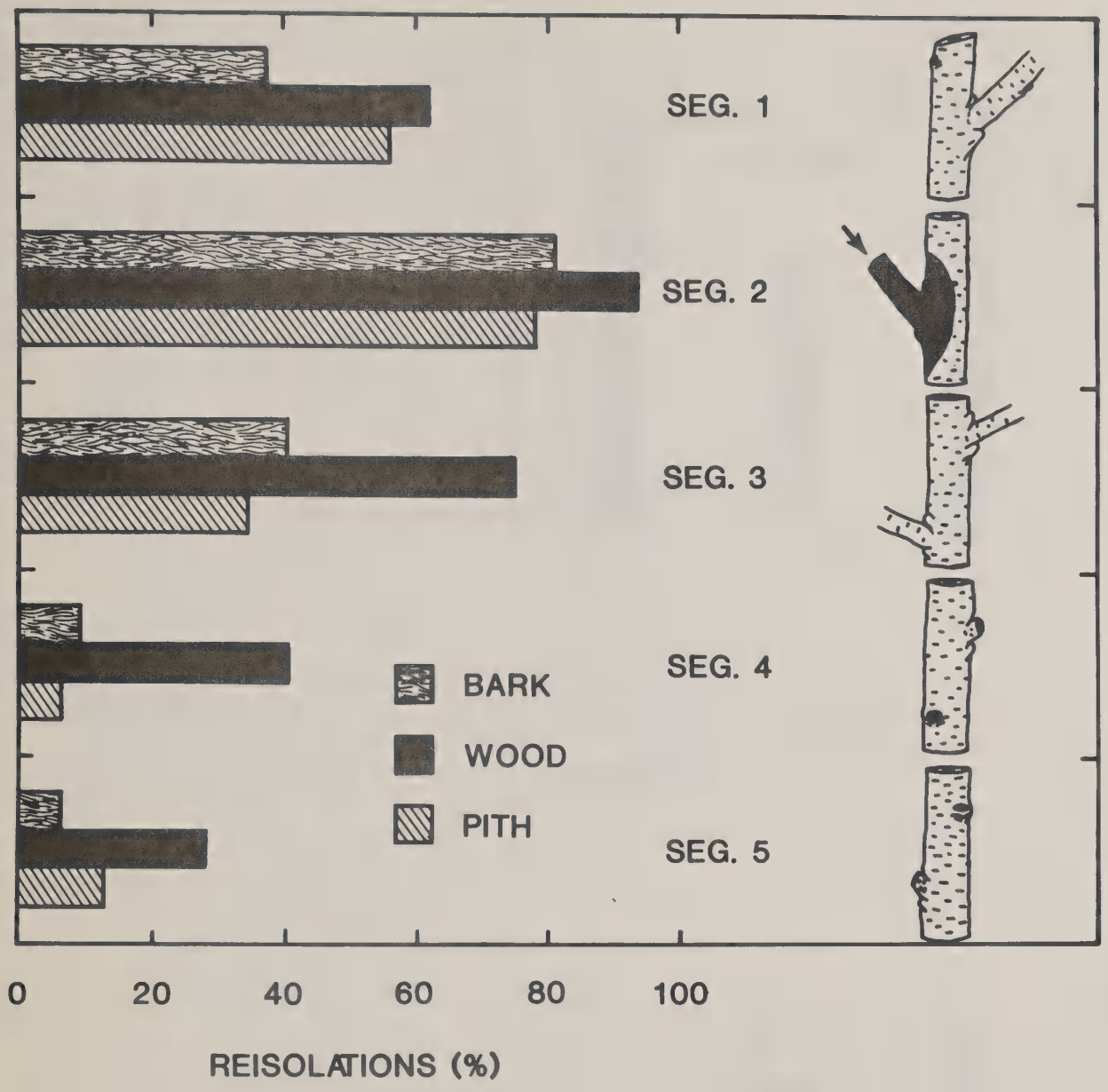


Fig. 1. Reisolation of Cytospora cincta from 32 peach seedlings in May 1984 following pruning-stub inoculation in November 1983. Each main stem was sectioned into five segments and separated into bark, wood, and pith components. The arrow represents the inoculation site; the shaded area represents cankered bark tissue.

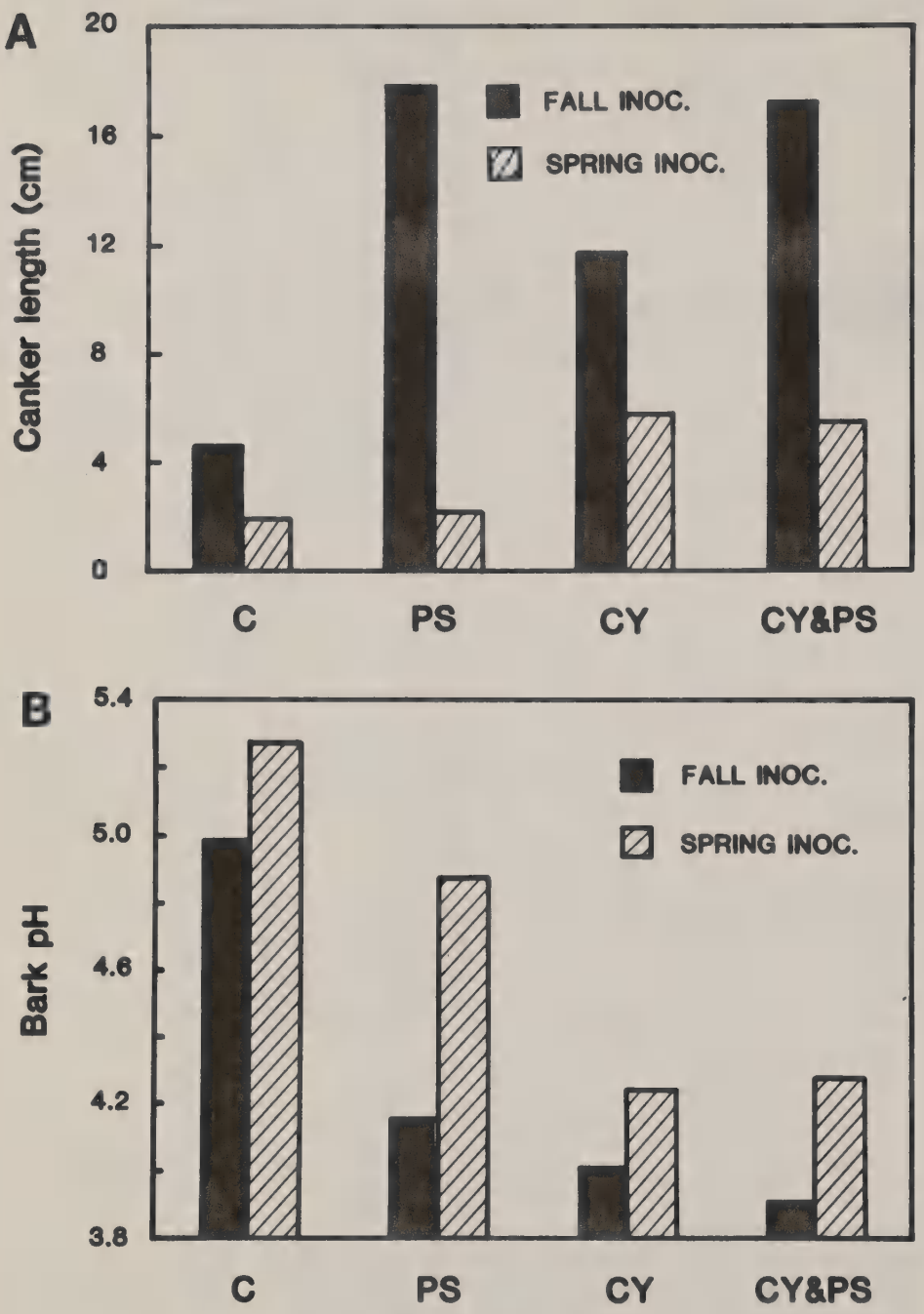


Fig. 2. Results of November 1983 and March 1984 inoculations of Cytospora cincta strain 4A and Pseudomonas syringae pv. syringae strain B-15+ rif 2 onto scaffold limbs of seven-yr-old peach trees ('Loring' on 'Lovell' rootstock). A, Length of bark cankers in April 1984; B, pH of inner bark 2-5 cm from the inoculation site. Each bar represents 18 inoculation sites.

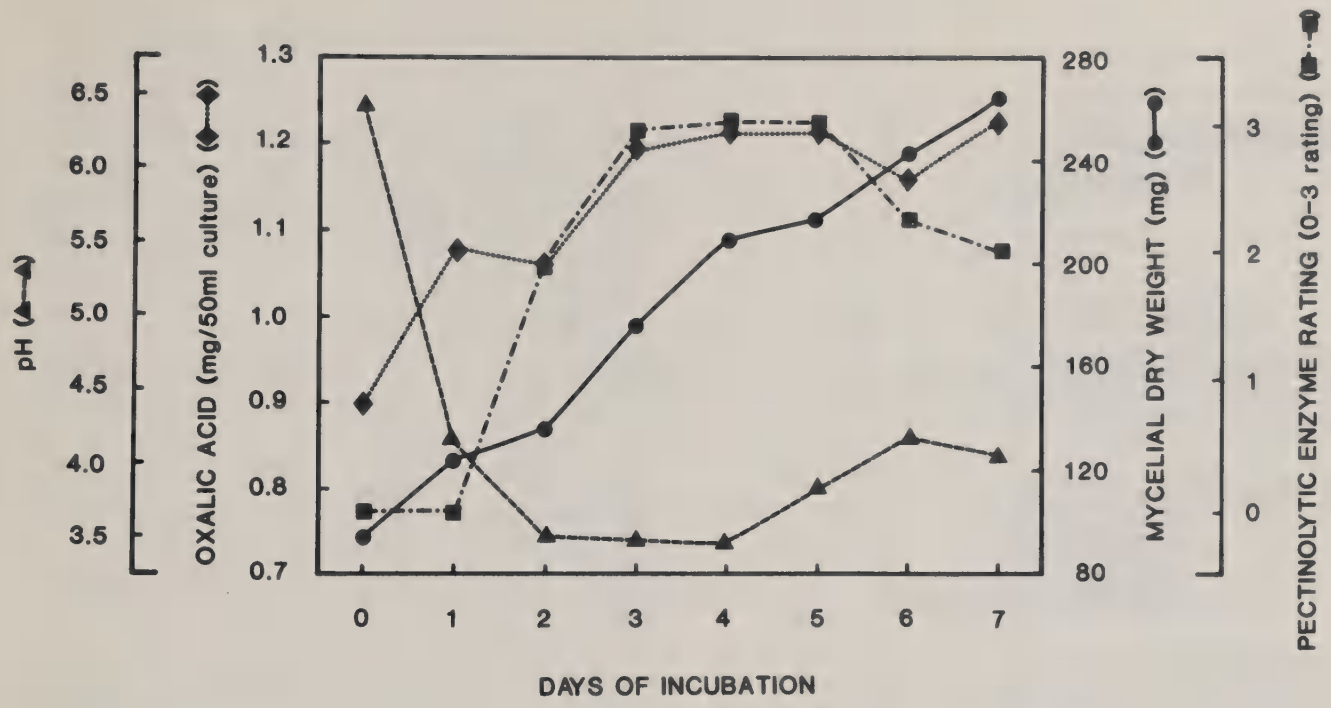


Fig. 3. Temporal relation of substrate pH, oxalic acid, and pectinolytic enzyme levels to mycelial growth of Cytospora cincta strain 9.2 in a liquid medium ccntaining 0.2% citrus pectin. Enzyme levels were rated on a 0-3 scale for maceration of carrot tissue after 24 hr incubation in culture filtrate. Means of nine replications.

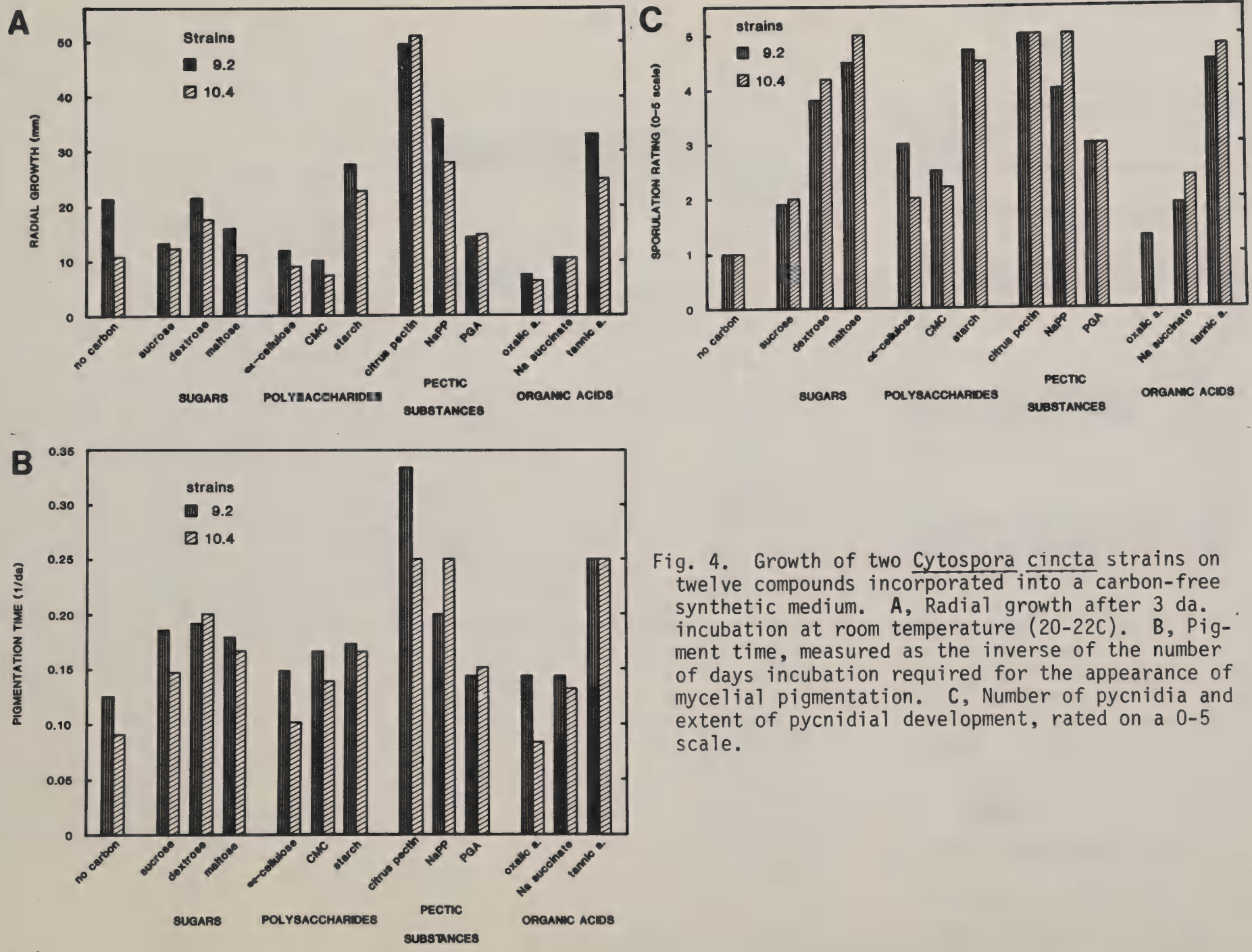


Fig. 4. Growth of two Cytospora cincta strains on twelve compounds incorporated into a carbon-free synthetic medium. A, Radial growth after 3 da. incubation at room temperature (20-22C). B, Pigment time, measured as the inverse of the number of days incubation required for the appearance of mycelial pigmentation. C, Number of pycnidia and extent of pycnidial development, rated on a 0-5 scale.

BACTERIAL CANKER OF STONE FRUITS

David F. Ritchie and Elke Endert
Department of Plant Pathology
North Carolina State University
Raleigh, NC 27695

Bacterial canker of stone fruits caused by Pseudomonas syringae pathovars has been one of the most intensively described bacterial diseases of deciduous fruit trees since it was first mentioned in the mid-1800's (1,2-9,25,41-46). Van Hall's report in 1902 is generally considered the first evidence a bacterium could cause cankers and gumming in fruit trees (1). In 1911, Griffin's work in Oregon established the bacterial origin of a gummosis disease of cherry limbs and that the bacterium also caused a blighting of dormant buds (21). In 1916, Barrett in California reported a similar canker disease occurring on apricot and termed it "bacterial gummosis" (41). Later, Goldsworthy and Smith reported a canker disease of plum describing it under the name of "sour-sap" (41). By 1930 numerous reports pointed out the close relationship between the organism causing bacterial canker of stone fruits, diseases on pear, citrus, avocado, and the bacterium causing lilac blight, thus they were all considered to be caused by Pseudomonas syringae (1,26). However, in England there were reports of a different, although similar bacterium causing cankers on cherry and plum that could infect through leaf scars in autumn without prior wounding (2,3,8,42-45). This bacterium was classified as Pseudomonas morsprunorum. It has also been reported to cause disease on cherry in North America (23,27).

Using the pathovar taxonomic system, there are currently two pathovars associated with bacterial canker of stone fruits, Pseudomonas syringae pv. syringae and Pseudomonas syringae pv. morsprunorum (20,37,47). P. syringae pv. syringae is relatively non-host specific and has been recorded as a pathogen or epiphyte on a wide range of plants (1,20,26). In contrast, P. syringae pv. morsprunorum is a specialized pathogen being restricted primarily to cherry and plum (3,8,20).

Many of the reports dealing with bacterial canker of stone fruits have extensively involved sweet and sour cherries (1,2-9, 21,26,27). Economic losses to cherry have varied depending on the part of the tree attacked. In Oregon, yield losses from dead buds may be as great as 80% (1). Without doubt, the greatest losses occur when major branches or the trunk are seriously infected which can result in death of the entire tree (1,19,23,25,31).

Symptoms on stone fruits, in addition to the canker and gummosis phase associated with these pathogens, are quite variable and seem to be dependent on host, geography, and environmental conditions

(1,8,19,40,41). Symptoms reported range from small cankers (1,23) to wilting and death of shoot tips, twigs and flowers (1), leaf spots (1,8,23), brown to black fruit lesions (1,23), death of dormant buds and fruit spurs (1,23), and dieback, and large extensive cankers that result in death of the entire tree to the soil-line (14,19,25,31). On apricot in eastern and southern Europe, bacterial canker has been associated with two major symptoms: one is the development of cankers of varying size, the second symptom is the dieback of branches or the entire tree with the term apoplexy used to describe this symptom (25). On peach in California, symptoms have been described as varying from bark cankers at pruning wounds, but more commonly surrounding spurs or buds, to the killing of scaffold branches or the entire young tree with the occurrence of gummosis (11). In the sour-sap phase, scaffold branches or the entire tree may suddenly collapse with the exudation of a sour smelling watery sap (19,41). The disease is most destructive, but not limited to 2 to 6-year-old trees. In peaches, plums and prunes, the disease is more common in sandy soils than soils of heavier texture, in cherries and apricots this relationship is not as apparent (19). In the southeastern U.S., bacterial canker has also been associated with the peach tree short life complex (14,31,39, 40).

For the remainder of this discussion, emphasis will be on bacterial canker and peach primarily as it relates to the peach tree short life (PTSL) complex in the southeastern U.S. The PTSL complex is characterized by the collapse and death of trees above the soil-line in late winter or spring that were apparently healthy the previous autumn (34). The two causes directly leading to tree death are considered to be freeze injury and bacterial canker caused by P. syringae pv. syringae (14,31,34,38-40). Additionally, this tissue is invaded and rapidly colonized by Cytospora spp. (34). Several factors have been demonstrated to predispose peach trees to bacterial canker and freeze injury (14,28,29,38-40). Thus it is very difficult, if not misleading, to discuss bacterial canker and P. syringae as single factors.

On apricot, it was found that simultaneous inoculation with P. syringae and Cytospora cincta caused more extensive cankers than when either pathogen was inoculated alone (35). P. syringae infection during the host vegetative period did not result in cankers or dieback, and when cankers did occur they were less than 10 cm in length; also, these cankers did not reactivate the following year (35). In in vitro and in vivo experiments we have detected antagonism of C. cincta toward P. syringae pv. syringae and data from preliminary experiments suggest antagonism is associated with a decrease in pH (Fig. 1). The three most common fungi detected in injured or dead peach bark were Cytospora cincta, Calosphaeria pulchella, and Schizophyllum commune. Of these, C. cincta was the only one to rapidly lower the pH to a level inhibitory to P. syringae pv. syringae (Fig. 1). The level of pH lethal to P. syringae pv. syringae seems to be between 4.2 and 4.6 (Fig. 2).

Injury from freezing is a common phenomenon in the northern peach growing regions of the U.S. where temperatures of 0 to -20 F are not uncommon. In the Southeast, minimum temperatures are seldom below +10 to 0 F and yet in some years thousands of trees are killed (32). It does not appear to be the absolute minimum temperature that causes death but instead the rapidity with which the temperature declines. In some years, major tree losses occur when the minimum temperature is only in the low 20's F (32).

If observed soon after the freeze injury occurs, the typical symptoms are split bark (although not always present), ease of bark separation from the wood, and retention of a normal bark color while the cambial area is brown to reddish-brown. In contrast, bark infected by P. syringae pv. syringae is completely brown, has a sour-sap odor, and is difficult to separate from the wood (40).

In 1960, bacterial canker was reported to be associated with peach tree death in several Southeastern states for the first time (31). A comparison of isolates showed a wide range of variation in virulence and although dormant bud, twig, branch and trunk infections were induced experimentally the massive death of limbs and trees observed in orchards did not occur (31). Variation among P. syringae pv. syringae isolates has been reported (10,18, 22,30). In addition, we have had poor success in detecting not only P. syringae pv. syringae but even P.syringae-like bacterial from injured trunk, limb, twig or bud tissue from North Carolina peach orchards. From 120 samples (representing 120 trees) in 1983, 2 samples had detectable fluorescent bacteria, both of which were virulent. In 1984, 54 samples of 226 (representing 198 trees) yielded detectable fluorescent bacteria and from these 54 samples 18 contained virulent P. syringae pv. syringae. Furthermore, we have yet to detect an isolate in North Carolina that is as virulent as isolates B-3A and B-15+ from California (18).

Because typical canker symptoms observed in orchards are often difficult to reproduce with P. syringae pv. syringae, and because P. syringae-like bacteria were detected in orchards without symptoms or in some cases were not detected following massive cankering and tree death, it has been suggested that other factors must be present for infection to occur and the disease to develop (14,15,17,18,31). In 1964, it was shown in California that soil fumigation could reduce the occurrence of the disease suggesting that factors in the soil contributed to bacterial canker development (19). Since this time, it has been shown that several factors can enhance the development of bacterial canker. Some of these include ring nematodes, early pruning, rootstock, soil pH, and freezing temperatures (14,28,29,31,39,40).

Several of the mechanisms hypothesized for P. syringae pv. syringae to induce disease are 1) production of a toxin designated syringomycin (13), 2) the bacterium acts as an ice nucleation center

causing a freezing of the tissue (38), and 3) the bacterium utilizes carbohydrates thus making the tree more sensitive to freeze injury (24).

The phytotoxin syringomycin was implicated in bacterial canker of peaches (13) and thought to cause necrosis in the vascular system (36). However, it has been reported that not all pathogenic strains produce toxin (10). Thus, simply the ability to produce toxin, although necessary for disease, may not be sufficient alone to cause disease. Even so in peach, a compound indistinguishable from syringomycin has been isolated from (36) as well as observed in infected host tissue (33).

In the mid-1970's, it was reported that P. syringae on surfaces of leaves could promote ice nucleation. This resulted in tissue containing P. syringae being injured at temperatures not injured when P. syringae was not present. It has been speculated that a similar situation may occur with P. syringae and peach (24,37).

The third proposed mechanism of action involves the utilization of starch and soluble sugars in dormant peach tissue. In 1966, Dowler and King reported that starch content was greatest in late fall, decreased rapidly until January, then remained constant until March; in contrast, soluble sugars were lowest in autumn, then increased as starch decreased (16). Starch content ranged from 15 to 4% while sugar was 2.5 to 21%. Total carbohydrate content changed little during dormancy except in the twigs and correlated with temperatures except in scaffold wood, trunk, or bark. In 1984, Klement et. al. reported experiments with apricot stem pieces that singly, neither P. syringae pv. syringae nor below freezing temperatures caused phloem necrosis; disease only occurred when bacterial infections preceded exposure of stem pieces to -5 C by 5 to 10 days (24). Bacterial multiplication prior to freezing utilized the sugars in the phloem tissue resulting in a 19 to 48% reduction in sugar. The infected phloem and cambium became more sensitive to freeze injury, thus the tissue became more susceptible to the pathogen so that bacterial numbers also increased (24).

Although bacterial canker and the causal bacterium P. syringae pv. syringae have been studied extensively from the descriptive to the molecular level, much remains unknown. The bacterium is ubiquitous, capable of existing as a parasite or an epiphyte in association with woody and herbaceous plants. At times, it has been associated with very extensive and destructive cankers of Prunus spp. and yet these same symptoms have been very difficult to reproduce under controlled conditions (1,31). A better knowledge of how the predisposition and stress factors effect and interact with the host as well as P. syringae pv. syringae may help explain the pathogensis of bacterial canker in stone fruits.

LITERATURE CITED

1. Cameron, H.R. 1962. Diseases of deciduous fruit trees incited by Pseudomonas syringae van Hall. Oregon Agric. Exp. Sta. Tech. Bull. 66. 64 pp.

2. Crosse, J.E. 1955. Bacterial canker of stone-fruits. I. Field observations on the avenues of autumnal infection of cherry. J. Hort. Sci. 30:131-142.

3. Crosse, J.E. 1956. Bacterial canker of stone-fruits. II. Leaf scar infection of cherry. J. Hort. Sci. 31:212-224.

4. Crosse, J.E. 1957. Bacterial canker of stone-fruits. III. Inoculum concentration and time of inoculation in relation to leaf-scar infection of cherry. Ann. Appl. Biol. 45:19-35.

5. Crosse, J.E. 1959. Bacterial canker of stone-fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Ann. Appl. Biol. 47:306-317.

6. Crosse, J.E. 1963. Bacterial canker of stone-fruits. V. A comparison of leaf-surface populations of Pseudomonas mors-prunorum in autumn on two cherry varieties. Ann. Appl. Biol. 52:97-104.

7. Crosse, J.E. 1965. Bacterial canker of stone-fruits. VI. Inhibition of leaf-scar infection of cherry by a saprophytic bacterium from the leaf surfaces. Ann. Appl. Biol. 56:149-160.

8. Crosse, J.E. 1966. Epidemiological relations of the Pseudomonad pathogens of deciduous fruit trees. Ann. Rev. Phytopathol. 4:291-310.

9. Crosse, J.E., and Garrett, C.M.E. 1966. Bacterial canker of stone-fruits. VII. Infection experiments with Pseudomonas mors-prunorum and P. syringae.

10. Currier, T.C., and Morgan, M.K. 1983. Plasmids of Pseudomonas syringae: No evidence of a role in toxin production or pathogenicity. Can. J. Microbiol. 29:2157-2163.

11. Davis, J.R., and English, H. 1969. Factors related to the development of bacterial canker in peach. Phytopathology 59:588-595.

12. Davis, J.R., and English, H. 1965. A canker condition of peach seedlings induced by chilling. Phytopathology 55:805-806.

13. DeVay, J.E., Lukezic, F.L., Sinden, S.L., English, H., Coplin, D.L. 1968. A biocide produced by pathogenic isolates of Pseudomonas syringae and its possible role in the bacterialcanker disease of peach trees. Phytopathology 58:95-101.

14. Dowler, W.M., and Petersen, D.H. 1966. Induction of bacterial canker of peach in the field. Phytopathology 56:989-990.

15. Dowler, W.M., and Weaver, D.J. 1975. Isolation and characterization of fluorescent Pseudomonads from apparently healthy peach trees. Phytopathology 65:233-236.

16. Dowler, W.M., King, F.D. 1966. Seasonal changes in starch and soluble sugar content of dormant peach tissues. Proc. Am. Soc. Hort. Sc. 89:80-84.

17. Endert, E., and Ritchie, D.F. 1984. Overwintering and survival of Pseudomonas syringae pv. syringae and symptom development in peach trees. plant Disease 68:468-470.

18. Endert, E., and Ritchie, D.F. 1984. Detection of pathogenicity, measurement of virulence, and determination of strain variation in Pseudomonas syringae pv. syringae. Plant Disease 68:677-680.

19. English, H., and DeVay, J.E. 1964. Influence of soil fumigation on growth and canker resistance of young fruit trees in California. Down To Earth 20:6-8.

20. Fahy, P.C., and Lloyd, A.B. 1983. Pseudomonas: The fluorescent Pseudomonads. In "Plant bacterial diseases a diagnostic guide", 141-188. (Ed. P.C. Fahy and G.J. Persley). Academic Press.

21. Griffin, F.L. 1911. A bacterial gummosis of cherries. Science (n.s.) 34:615-616.

22. Gross, D.C., Cody,Y.S., Proebsting, E.L.,Jr., Radamaker, G.K., and Spotts, R.A. 1984. Ecotypes and pathogenicity of ice-nucleation-active Pseudomonas syringae isolated from deciduous fruit tree orchards. Phytopathology 74:241-248.

23. Jones, A.L. 1971. Bacterial canker of sweet cherry in Michigan. Plant Dis. Reptr. 55:961-965.

24. Klement, Z., Rozsnyay, D.S., Balo, E., Panczel, M., Pryileszky, G. 1984. The effect of cold on development of bacterial canker in apricot trees infected with Pseudomonas syringae pv. syringae. Physiol. Plant Pathol. 24:237-246.

25. Klement, Z. 1977. Bacterial canker and dieback of apricot (Pseudomonas syringae van Hall). EPPO Bull. 7:57-68.

26. Latorre, B.A., and Jones, A.L. 1979. Evaluation of weeds and plant refuse as potential sources of inoculum of Pseudomonas syringae in bacterial canker of cherry. Phytopathology 69:1122-1125.

27. Latorre, B.A., and Jones, A.L. 1979. Pseudomonas morsprunorum, the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with P. syringae. Phytopathology 69:335-339.

28. Lowsberry, B.F., English, H., Moody, E.H., and Shick, F.J. 1973. Criconemoides xenoplax experimentally associated with disease of peach. Phytopathology 63:994-997.

29. Lowsberry, B.F., English, H., Noel,G.R., and Shick, F.J. 1977. Influence of Nemaguard and Lovell rootstocks aand Macropothonia xenoplax on bacterial canker of peach. J. Nematol. 9:221-224.

30. Perlasca, G. 1960. Relationships amoung isolates of Pseudomonas syringae pathogenic on stone fruit trees. Phytopathology 50:889-899.

31. Petersen, D.H., and Dowler, W.M. 1965. Bacterial canker of stone fruits in the southeastern states. Plant Dis. Reptr. 49:701-702.

32. Prince, V.E. 1966. Winter injury to peach trees in central Georgia. J. Am. Soc. Hort. Sc. 88:190-196.

33. Paynter, V.A., and Alconero, R. 1979. A specific fluorescent antibody for detection of syringomycin in infected peach tissues. Phytopathology 69:493-496.

34. Ritchie, D.F., and Clayton, C.N. 1981. Peach tree short life: A complex of interacting factors. Plant Disease 65:462-469.

35. Rozsnyay, Zs.D., and Klement, Z. 1977. Simultaneous infection by Pseudomonas syringae van Hall and Cytospora cincta Sacc. on apricot. EEPO Bull. 7:81-84.

36. Sinden, S.L., DeVay, J.E., and Backman, P.A. 1971. Properties of syringomycin, a wide spectrum antibiotic and phytotoxin produced by Pseudomonas syringae, and its role in the bacterial canker disease of peach trees. Physiol. Pl. Pathol. 1:199-213.

37. Skerman, V.B.D., McGrown, V., and Sneath, P.H.A. 1980. Approved list of bacterial names. Internat. J. Systematic Bacteriol. 30:225-420.

38. Weaver, D.J. 1978. Interaction of Pseudomonas syringae and freezing in bacterial canker on excised peach twigs. Phytopathology 68:1460-1463.

39. Weaver, D.J., and Wehunt, E.J. 1975. Effect of soil pH on susceptibility of peach to Pseudomonas syringae. Phytopathology 65:984-989.

40. Weaver, D.J., Wehunt, E.J., and Dowler, W.M. 1974. Association of tree site, Pseudomonas syringae, Criconemoides xenoplax, and pruning date with short life of peach trees in Georgia. Plant Dis. Reptr. 58:76-79.

41. Wilson, E.E. 1933. Bacterial canker of stone-fruit trees in California. Hilgardia 8:83-123.

42. Wormald, H. 1930. Bacterial diseases of stone-fruit trees in Britain. II. Bacterial shoot wilt of plum trees. Ann. Appl. Biol. 17:725-748.

43. Wormald, H. 1931. Bacterial diseases of stone-fruit trees in Britain. III. The symptoms of bacterial canker in plum trees. J. Hort. Sci. 9:239-259.

44. Wormald, H. 1932. Bacterial diseases of stone-fruit trees in Britain. IV. The organism causing bacterial canker of plum trees. Trans. Brit. Mycol. Soc. 17:157-169.

45. Wormald, H. 1938. Bacterial diseases of stone-fruit trees in Britain. VII. The organisms causing bacterial diseases in sweet cherries. J. Pomol. 16:280-290.

46. Wormald, H. 1942. Bacterial diseases of stone-fruit trees in Britain. VIII. Bacterial canker of peach. Trans. Brit. Mycol. Soc. 25:246-249.

47. Young, J.M., Dye, D.W., Bradbury,J.F., Panagopoulos, C.G., and Robbs, C.F. 1978. A proposed nomenclature and classification for plant pathogenic bacteria. New Zealand J. Agric. Res. 21:153-177.

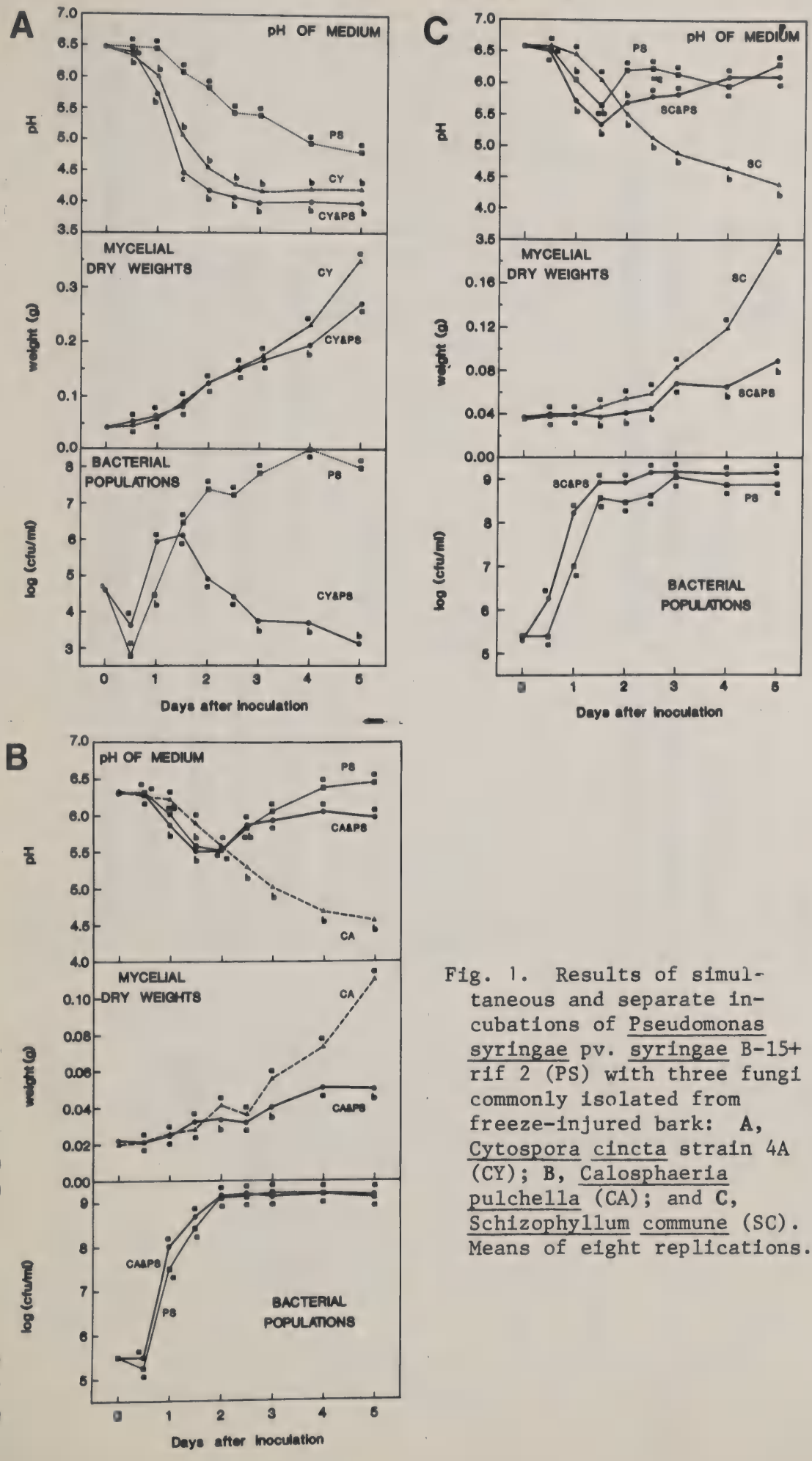


Fig. 1. Results of simultaneous and separate incubations of Pseudomonas syringae pv. syringae B-15+ rif 2 (PS) with three fungi commonly isolated from freeze-injured bark: A, Cytospora cincta strain 4A (CY); B, Calosphaeria pulchella (CA); and C, Schizophyllum commune (SC). Means of eight replications.

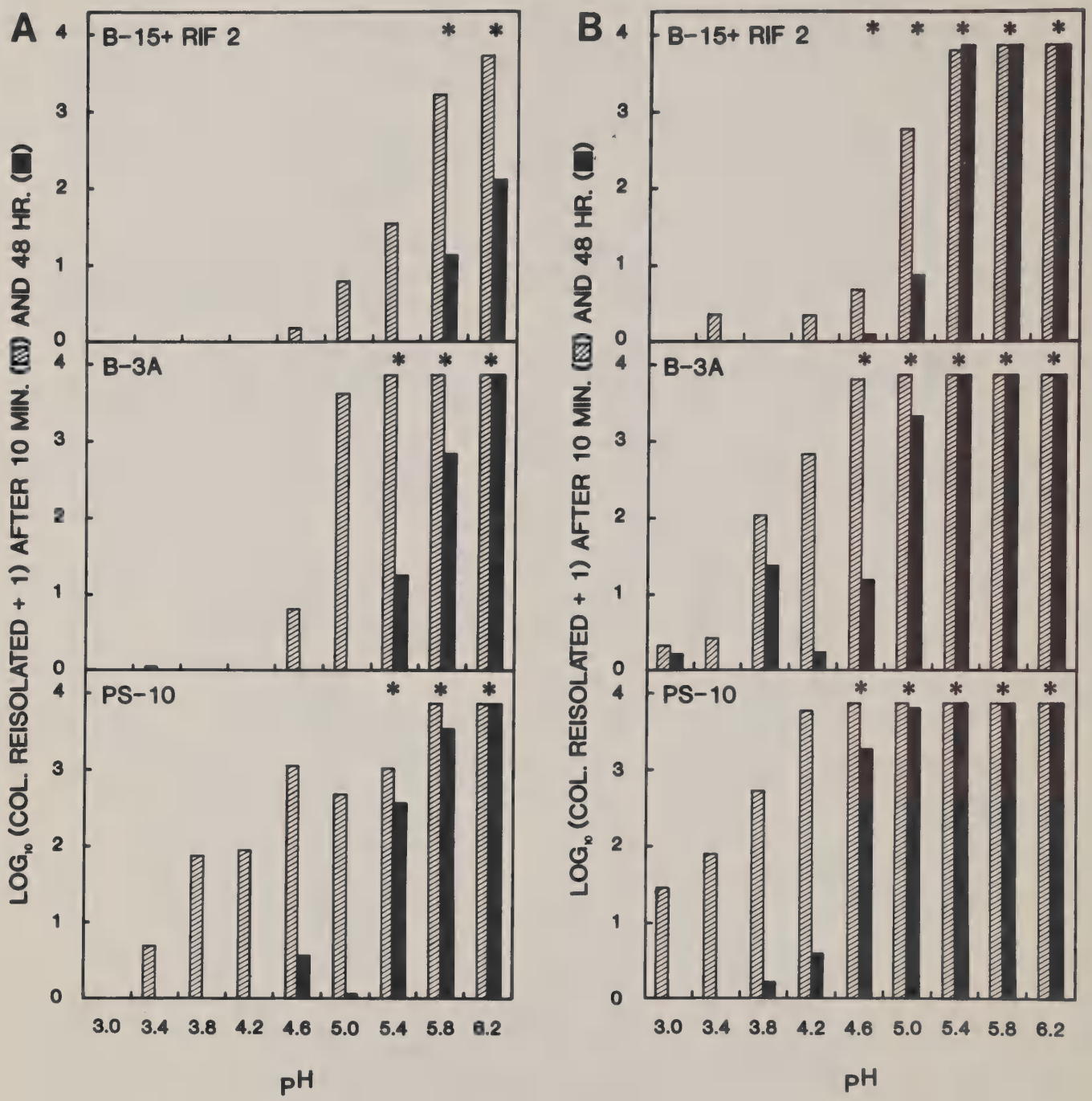


Fig. 2. Effect of pH on the survival of Pseudomonas syringae pv. syringae. Strain B-15+ was originally isolated from almond, whereas strains B-3A and PS-10 were isolated from peach. **A**, citric acid/sodium citrate buffers, 0.1M; **B**, oxalic acid, 0.01M, adjusted with varying concentrations of KOH. Means of five replications. Asterisks indicate reisolation after two weeks.

CANKERS AND DECLINE OF STONE FRUIT

C. L. Parish, Plant Pathologist
USDA, ARS, Tree Fruit Research Laboratory
1104 N. Western Avenue, Wenatchee, WA 98801

The topic of this discussion is virus and virus-like cankers and decline of stone fruits. However, cankers and/or decline caused by prunus necrotic ringspot virus will not be discussed as this topic is covered adequately in other papers in this session. First, decline and/or cankers of cherries (Prunus avium) will be discussed followed by those of peach (P. perisca) and finally apricot (P. armeniaca).

Cherry

Van decline. When 'Van' is grown on Mahaleb (P. mahaleb) rootstock about 1/3 of the trees will start to decline at the fifth year. This decline is very similar to the decline observed with partial girdling. The first observable symptoms are reduced vigor in the spring and summer with heavy fruit set and pre-mature leaf reddening in late August to early September. The affected trees continue to decline with time (2-3 years), and the trees soon become so weakened, they are no longer an economical unit or they die. The root system is usually poorly developed. Generally there is a marked swelling of the bark above the graft union.

This disorder is controlled by not growing 'Van' on Mahaleb rootstock. 'Van' decline on Mahaleb rootstock is believed to graft incompatibility. No evidence exists to indicate the decline is induced by a pathogenic agent. The fact that only a percentage of the trees decline can be explained by genetic variation or by a pathogenic agent. At present the issue is not resolved, in my opinion, as to why only 'Van' reacts on Mahaleb and not 'Bing' or 'Lambert'. The disorder has not been reported to be transmissible, but it may be in the future.

X-disease. In sweet cherry cultivars X-disease mycoplasm induces a "little cherry-type" symptom (small, light-colored fruits). Cherry trees grown on Mahaleb rootstock also decline and die when infected with X-disease mycoplasm. The reaction is very similar to pears (Pyrus communis) infected with pear-decline mycoplasm grown on certain oriental rootstocks. Cherry trees grown on Mahaleb rootstock are self-rouging when infected with X-disease Mycoplasm. The scion (sweet cherry cultivar) becomes infected, and as the mycoplasm moves down to the graft union, an incompatibility reaction occurs. The tree declines and soon dies. It appears the Mahaleb rootstock is hypersensitive to the X-disease mycoplasm just as certain oriental pear rootstocks appear to be hypersensitive to pear decline mycoplasm.

Black Canker. Black canker was first described in Oregon and since has been reported in Washington and the Okanagan Valley of British Columbia. Sweet cherry is the only known host. 'Napoleon' is the most severely affected cultivar, but severe symptoms have been noted also on 'Bing', 'Van', 'Republican' and 'Deacon'.

The most distinctive symptoms of this disease are the rough black cankers occurring on the scaffold limbs and branches of affected trees. The symptoms may first

appear as gumming at the base of a spur or as a dying spur with a small canker at its base. Cankers start as swollen areas on branches and twigs. The bark splits lengthwise along these swollen areas forming rough, thickened ulcer-like wounds. In some instances, cankers may completely girdle a twig or branch, causing dieback. Affected trees are generally debilitated.

This disease has been transmitted by grafting. After inoculation 2 to 3 years are required for symptoms to develop. Nursery certification programs, increased grower awareness of virus problems, and the apparent absence of vectors have combined to make this potentially economically important disease a rarity. It still occurs in Washington in some isolated foothill orchards.

Tomato bushy stunt. This virus was first detected in cherry in 1963 in Ontario by Allen and Davidson (1967) who reported leaf and fruit symptoms. The only other report of tomato bushy stunt virus (TmBSV) in fruit trees in North America was by Hansen (1973) in British Columbia where twig and limb symptoms were noticed. TmBSV induces necrosis in leaf veins. When the necrosis occurs in a mid-rib, a twist or kink of the leaf results that resembles cherry twisted leaf. On small branches kinks (up to 30°) can occur. This is usually associated with gumming from or very close to the kink. In some instances gum boils and stem pitting occur on large limbs and the trunk. One diagnostic symptom is usually the flower or fruit stem is shorter than normal and pitting occurs in the fruit.

TmBSV is readily isolated and purified (Allen and Davidson 1967 and Hansen, personal communication). Graft and bud transmission is difficult (Hansen, personal communication).

Detrimental canker. Detrimental canker has been reported in Europe (Blattny 1962; Schmid 1968; Kunz, et al 1983) but not in North America. Detrimental canker is induced by petunia astroid mosaic virus (PAMV) (Koenig and Kunz 1982). PAMV induces necrotic areas on the leaf blade which usually result in distortion of the leaf. If the necrosis occurs in a leaf mid-rib or petiole, it frequently causes a severe twist. Necrosis can occur also in new shoots usually along one side. The normal growth appears to be interrupted and shoots bend or twist as a result of the necrosis. In a few instances the shoot may die. Bark splitting is observed in or near the area of the twist.

As with tomato bushy stunt PAMV induces a fruit deformation and internal necrosis (Schmid 1968). PAMV is seriologically related to tomato bushy stunt. It is possible detrimental canker and tomato bushy stunt is the same disease caused by two sero-types.

Bark splitting in 'Montmorency' cherry. Bark splitting was first reported by Cameron in 1954. The symptoms first start as brown streaks on new shoots in the summer. The bark splits along these streaks and gumming occurs. The symptoms reappear each year on the new growth (Cameron 1976). The affected shoots tend to bend away from the trunk. With time, the affected areas appear somewhat flattened and weak.

In apricot, the symptoms are similar to those of apricot gummosis in that numerous gum-pockets occur. However, the causal agent of bark splitting does not incite symptoms on shiro-fugen flowering cherry (P. serrulata), an indicator of prunus

necrotic ringspot virus, and prune dwarf virus; the causal agent of apricot gummosis does incite symptoms on flowering cherry. The causal agent of bark splitting is transmitted by grafting, but there is no evidence of natural spread (Cameron 1976).

Rough bark of sweet cherry. This disorder has been reported only in California. The symptoms first appear as raised blisters on the bark which rupture and exude gum. These cankers enlarge with time (about 3 years) (Nichols 1976). The symptoms are very similar to cherry black canker except the causal agent of cherry rough bark also incites symptoms of prunus necrotic ringspot virus, and it indexes positive on shiro-fugen flowering cherry (an indicator host for both prunus necrotic ringspot virus and prune dwarf virus) (Nichols 1976).

It is not known if the causal agent is a single entity (e.g. a strain of prunus necrotic ringspot virus) inciting both types of symptoms or whether it consists of two entities (e.g. black canker plus prunus necrotic ringspot virus).

Peach

Bunchy stunt. Peach trees infected with prunus necrotic ringspot virus (PNRSV) or with prune dwarf virus (PDV) exhibit reduced growth and vigor. If peach trees are infected with both PNRSV and PDV, the reduction of growth is more than either virus alone can induce and is more than additive. The inter-nodes of the new shoot growth are quite short in the spring; hence the name, bunchy stunt. If the infected trees are grown in areas where the summers are hot (approaching 100°F) the inter-nodes in the new growth become nearly normal and the symptoms of bunchy stunt are not as apparent.

X-Disease. X-disease in peach can induce reduced vigor, decline and/or death depending on the strain of the mycoplasm. The first visible symptoms on peach early in summer are light-colored water-soaked areas in the leaves; sometimes a red color may be associated with it. The water-soaked areas soon die and fall out. At this point, the affected leaves are attached loosely and fall readily from the tree. The tree goes into a decline and usually dies.

X-disease is field-spread and vectored by several leafhoppers. Tree removal is recommended.

X-disease of peach and arsenic toxicity are sometimes confused. Arsenic spots are interveinal and along the leaf margins. They are round, brownish-black, and more regular in shape. Defoliation frequently occurs. The symptoms are more general throughout the tree. X-disease spots are irregular, and the leaves tend to roll and curl downwards. The basal leaves are affected first. Usually one area or limb of the tree is affected more severely than another. The next year X-affected limbs may be dead. Arsenic-affected limbs do not usually die. Also, peaches on X-affected limbs tend to wither and drop, whereas peaches on arsenic-affected limbs mature. Leaf analysis may be the best way to distinguish X-disease from arsenic toxicity.

Stem grooving in nectarine and peach. This disorder was first observed in a nectarine and peach orchard south of Yakima, Washington in 1969. The first

noticeable symptoms are creases or small grooves in 2-4-year-old limbs. The grooves get longer and deeper with age, about 1 cm deep and 10-15 cm long. The grooves extend into the wood, and the cambium in the bottom of the grooves appears dead. The bark is somewhat thickened but not as thick as it is with prunus stem pitting. The symptoms are reminiscent of apple stem grooving or apple flat limb.

The first attempts at transmission were unsuccessful and some investigators had attributed the stem grooving to winter injury or a varietal sport. This disorder or one similar to it was transmitted by Rosenberger and Jones (1976) in Michigan. The disorder in Yakima was later transmitted (Parish, unpublished data). No evidence exists for natural spread.

Apricot

Apricot gummosis. Apricot gummosis is a graft-transmissible disease induced by a strain of prune dwarf virus (PDV). The 3-4-year-old wood gums; older wood has gum cankers and splits or cracks. The overall appearance is rough, cracked bark with gum cankers. The tree is of low vigor but usually will not die.

Apricot ringpox. Apricot ringpox in certain apricot cultivars induce pox-like 'scabs' on the fruits, as well as leaf and twig symptoms. The leaf symptoms start as white spots that fall out. Purplish lesions can be observed on the leaf petioles and green shoots. On the older woody twigs small lesions or bark splits are observed.

Literature Cited

Allen, W.R. and T.R. Davidson. 1967. Tomato bushy stunt virus from prunus avium L. Can. J. Bot. 45:2375-2383.

Blattny, C. 1962. Detrimental canker, a virus disease of cherry. Rostlinna Vyroba 8:577-588.

Cameron, H.R. 1954. A bark splitting virus disease of 'Montmorency' sour cherry. Phytopathology 44:484. (Abstract)

Cameron, H.R. 1976. Bark splitting in 'Montmorency' cherry. p. 191-192. In Virus diseases and noninfections disorders of stone fruits in North America. USDA Agric. Handbook 437.

Hansen, A.J. 1975. Differences between twisted leaf and tomato bushy stunt virus in sweet cherry. Acta Hort. 44:55-57.

Koenig, R. and L. Kunze. 1982. Identification of tombusvirus isolates from cherry in southern Germany as petunia asteroid mosaic virus. Phytopath. Z. 103:361-368.

Kunze, L., C. Krause and R. Koenig. 1983. Occurrence of a virus-induced twig necrosis on sweet cherries in southern Germany. Nachrichtenbl. Deut. Pflanzenschutzd. 35(2):17-21.

Nichols, C.W. 1976. Rough bark of sweet cherry. p. 240-241. In Virus diseases and noninfectious disorders of stone fruits in North America. USDA Agric. Handbook 437.

Rosenberger, D.A. and A.L. Jones. 1976. A graft-transmissible agent associated with bark- and wood-grooving disease of peach and nectarine. Phytopathology 66:729-730.

Schmid, G. 1976. Investigation on detrimental canker of sweet cherries. Tagungsber. Dtsch. Akad. Landw. Wiss. Berlin 97:155-163.

THE RELATIONSHIP BETWEEN PRUNUS NECROTIC RINGSPOT VIRUS AND WINTER INJURY/CYTOSPORA CANKER IN EIGHT PENNSYLVANIA PEACH ORCHARDS: PRELIMINARY RESULTS

B. A. Jaffee[1], C. A. Powell[2], L. B. Forer[2], and K. D. Hickey[1]

Winter injury and Cytospora canker are major causes of peach tree decline in Pennsylvania. Susceptibility to winter injury and Cytospora may reflect the general health of the tree, i.e., previously weakened trees may be more susceptible. A survey was conducted to determine if a relationship existed between infection with Prunus Necrotic Ringspot Virus (PNRSV) and canker of peach trees in Pennsylvania.

Eight commercial peach orchards (9-17 years old: 4 Loring, 3 Redhaven, 1 Garnet Beauty) in Adams and Franklin Counties were rated for canker (none, moderate, severe) on the trunk or main scaffolds and were sampled for PNRSV. When possible, 10 trees in each canker category were sampled per orchard. Canker ratings were made in early April 1984. Five terminal shoots per tree were collected April 10 to April 12 (pre-bloom). Shoots were randomly selected from canker-rated trees unless the tree was rated "moderate". If the tree was rated "moderate", shoots were collected from cankered scaffolds. The base of each shoot was placed in water and the expanding buds were assayed for PNRSV by ELISA and by an immun-blot assay (indirect). Thirty of the 206 trees sampled were positive for PNRSV (Table 1). The ratio of positive trees: total trees was 5:104, 6:74, and 19:23 for Loring, Redhaven, and Garnet Beauty, respectively.

In the Loring and Redhaven orchards, no apparent relationship existed between canker and PNRSV as determined by Chi^2 analysis ($P = 0.54$ and 0.60, respectively). In the one Garnet Beauty orchard, an apparent relationship was detected ($P = 0.03$). These data do not support the hypothesis that PNRSV is a major factor contributing to tree decline in Pennsylvania.

[1]Department of Plant Pathology, The Pennsylvania State University, Fruit Research Laboratory, Biglerville, PA 17307.

[2]The Pennsylvania Department of Agriculture, Bureau of Plant Industry, Harrisburg, PA 17110.

Table 1. Relationship between canker and Prunus Necrotic Ringspot Virus in eight Pennsylvania peach orchards (206 trees sampled in spring 1984).

Cultivar	Canker Rating	# trees		% trees	
		-[a]	+[a]	-[a]	+[a]
All Cultivars (n=206)	None	73	6	92	8
	Mod	57	13	81	19
	Severe	46	11	81	19
P=0.06[b]					
Loring (n=109)	None	31	1	97	3
	Mod	37	3	93	7
	Severe	36	1	97	3
P=0.54					
Redhaven (n=74)	None	40	4	91	9
	Mod	18	2	90	10
	Severe	10	0	100	0
P=0.60					
Garnet Beauty (n=23)	None	2	1	67	33
	Mod	2	8	20	80
	Severe	0	10	0	100
P=0.03					
All Cultivars minus Garnet Beauty (n=183)	None	71	5	93	7
	Mod	55	5	92	8
	Severe	46	1	98	2
P=0.39					

[a]Negative or positive for PNRSV as determined by ELISA.

[b]Based on Chi-square analysis.

CANKERS CAUSED BY PRUNUS NECROTIC RINGSPOT VIRUS IN DECLINING PEACH ORCHARDS IN GEORGIA

J. M. Wells, H. C. Kirkpatrick and C. L. Parish
USDA, ARS
New Brunswick, NJ, Mukilteo, WA, and Byron, GA

Abstract

Prunus necrotic ringspot virus (PRSV) causes transitory foliar symptoms and persistent necrosis on cultivated peach trees in Georgia. Persistent symptoms include dieback of terminal twigs, bark necrosis, cankers, bark and trunk splitting, and the production of vigorous root sprouts. In a state-wide survey of peach orchards in Georgia, PRSV was detected in trees in Peach and Houston counties. Highest percentage of trees in PRSV, as determined by Shirofugen and ELISA assays, occurred in those with bark necrosis and cankers--82% in Houston county and 37% in Peach county.

Prunus necrotic ringspot virus (PRSV) is one of the most commonly encountered viruses in cultivated species of Prunus. The virus occurs in a variety of strains causing diversity in the type of symptoms on a standard host range (5). Prunus RSV may also occur in combination with other viruses such as Prune dwarf virus (PDV) and is recognized as a common contaminant of stone fruit virus cultures (6). In peach trees PRSV may cause initial shock symptoms including leaf chlorosis, necrosis and deformation and partial dieback of twigs. Some strains may then become latent, others may cause severe necrosis and mortality, particularly in susceptible cultivars.

A limited survey of peach cultivars in central Georgia in 1953 failed to detect the presence of PRVS (4). In the 1970's, however, observations were frequently made by the second author (H. C. Kirkpatrick) of partial or complete trunk necrosis, and cankering twig dieback on peach trees undergoing slow and progressive decline in Peach and Houston counties. Foliar symptoms were not generally observed. Our investigation, therefore, was initiated to determine the possible involvement of PRSV in slow decline of peach trees.

Materials and Methods

Virus sources were obtained from P. W. Cheney of the Fruit Research Lab, Wenatchee, WA, and by the second author from naturally-infected trees in Peach and Houston counties, GA. Strains were screened against the presence of other contaminating stone fruit viruses, and kept on bud-inoculated peach seedlings, Prunus persica (L.) Batsch cv 'Elberta', in the greenhouse.

Symptomology of three strains, severe PRSV #TR4T16 and #R19T11, and mild PRSV #R19T8 was determined by bud inoculation on a group of Prunus host plants including Chickasaw plum, P. augustifolia Marsh; common plum cv 'Mariana' (interspecific hybrid); wild black cherry, P. serotina Ehrh; Mahaleb cherry, P. mahaleb L.: Shirofugen cherry, P. serrulata Lindl. cv 'Shirofugen'; Japanese cherry, P. serrulata cv 'Kwanzan'; Nanking cherry, P. tomentosa Thunb; Jordanola almond, P. dulcis (Mill,) D. A. Webb; and peach, P. persica cv 'Elberta', 'Lovell' and 'Redskin'. Herbaceous hosts included cucumber, Cucumis sativus L., and Butternut squash, C. maxima, Dcne.

Trees from orchards in peach-growing counties of Georgia were indexed for PRSV on Shirofugen flowering cherry trees and by the enzyme-linked immunosorbant assay (ELISA). The Shirofugen bioassay was based on necrotic reactions from 3 buds per tree (3). Indexing always included a positive check obtained from known PRSV source trees in the greenhouse. Duplicate samples of bud tissue was tested serologically by ELISA (1). Anti-serum was obtained from R. W. Fulton, Madison, Wisconsin. Samples were also tested for PDV with anti-serum PVAS-33 from the American Type Culture Collection. Coating and conjugate gamma-globulin were used at 1:1000 and 1:800 dilution, respectively. Sample trees positive in the Shirofugen bioassay or in the ELISA test were considered infected with PRSV.

Results

The sources of PRSV were free of PDV, and caused symptoms typical of PRSV on a range of host plants. Foliar chlorotic rings were produced on common plum, wild black cherry, Mahaleb cherry, Nanking cherry, Jordanola almond and peach (Table 1). On Jordanola almond and peach foliar symptoms were evident only in the first year after inoculation. Older peach trees developed necrosis of terminal twings (dieback), bark cankers, and in many cases severe necrotic splittings of the trunk (Table 2). On Shirofugen cherry typical necrotic lesions developed within 2 weeks around bud graft sites. There were no symptoms on Chickasaw plum or on Japanese cherry.

On herbaceous hosts, all strains of PRSV caused local lesions on cotyledons of cucumber and systemic mosaic on leaves. Reactions on butternut squash were typical of PRSV and not PDV (Table 2).

Based on a limited state-wide survey of peach orchards, PRSV was found only in Peach and Houston counties in central Georgia (Table 4). Positive Shirofugen and ELISA reactions were associated with symptomless trees in orchards undergoing slow decline, in trees with dieback of terminal twigs only, and in trees with bark necrosis and cankers. The highest percentage of trees with PRSV occurred in the group with cankers -- 82% in Houston county and 37% in Peach county. In contrast, PRSV was detected in 42% and 16%, respectively, of symptomless trees. No PRSV was detected in Brooks, Talbot, McDuffee and Morgan counties, in which were found no trees with canker (7).

Discussion

Prunus RSV is established in peach orchards in central Georgia. The incidence of PRSV was associated with orchards having trees in various stages of decline including cankers of scaffold limbs and trunks. The highest incidence of PRSV was in trees with cankers.

Prunus RSV is calculated to cause significant losses in peach orchards in California (2). Although such estimates may not be fully applicable to growing conditions and cultural practices in Georgia and in southeastern U. S., it is clear that PRSV is a

disease that should be controlled. Screening of bud wood sources and nursery stock against PRSV should be rigorously practiced. Variability in cultivar susceptibility to PRSV was evident in our replicated tests. Cultivars of peaches commonly planted in the southeast should be tested for resistance to PRSV. Screening for disease resistance in advance breeding selections should include a test for susceptibility to PRSV.

Literature Cited

1. Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate methods of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-484.

2. Heaton, C. R., Ogawa, J. M., and Nyland, G. 1981. Evaluating economic losses caused by pathogens of fruit and nut crops. Plant Disease 65:886-888.

3. Helton, A. W. 1962. Relative merits of Shirofugen and peach trees as indicators for Prunus Ringspot Virus in prune trees. Phytopathology 52:846-849.

4. KenKnight, G., and Jones, J. F. 1953. Apparent absence of the ring spot virus in the peach variety collection at the U. S. Horticultural Station, Fort Valley, Georgia. Plant Dis. Rep. 37:346.

5. Nyland, G., Gilmer, R. M., and Moore, J. D. 1974. Prunus ring spot group. In Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. U. S. Dept. Agric., Agric. Handbook 437. 433 pp.

6. Parker, K. G., and Cochran, L. C. 1951. Similarities of symptoms produced by the viruses causing ring spot of peach and necrotic ring spot of sour and sweet cherry. Phytopathology 41:942.

7. Wells, J. M., Kirkpatrick, H. C., and Parish, C. L. 1985. Symptomology and incidence of Prunus necrotic ringspot virus in peach orchards in Georgia. Plant Disease (in press).

Table 1. Symptoms of PRSV on peach and other Prunus hosts in Georgia.

Host*	Symptoms	Incubation
Chickasaw plum	None	-
Common plum	Foliar chlorotic rings	7 mos
Wild black cherry	Foliar chlorotic rings, dehiscence	7 mos
Mahaleb cherry	Foliar chlorotic rings	11 mos
Shirofugen cherry	Bark necrosis	2 wks
Japanese cherry	None	-
Nanking cherry	Foliar chlorotic rings, mottle	2 mos
Jordanola almond	Foliar chlorotic rings, (transitory)	2 mos
Peach cv. 'Elberta'	Foliar chlorotic rings, bark necrosis	2 mos

* One year old seedlings inoculated by budding from known PRSV source trees.

Table 2. Percent of bud-inoculated peach trees showing specific symptoms of severe strain PRSV in 5 Years.

	Cultivar *		
Symptoms	June Gold	Mayflower	Maygold
No symptoms	0	7	20
Foliar chlorosis	100	53	50
Foliar necrosis	100	47	50
Twig dieback	100	40	60
Basal sprouts	64	77	40
Bark cankers	18	77	20
Bark splitting	28	77	20
Tree stunting	36	27	0

* Based on 28 June Gold, 30 Mayflower and 10 Maygold trees inoculated Aug.-Sept. 1974.

Table 3. Tests for absence of prune dwarf virus contaminants in Prunus necrotic ring spot (PRSV) source material.

RSV			Symptoms *	
Strain	Source	Reaction on peach	Cucumber	Butternut squash
TR4T16	Wenatchee, WA	Severe	Systemic mosaic	Chlorotic blotches
W1161	Peach Co., GA	Severe	Systemic mosaic	Chlorotic blotches
R19T11	Houston Co., GA	Severe	Systemic mosaic	Chlorotic blotches
R19T12	Houston Co., GA	Severe	Systemic mosaic	Chlorotic blotches
R19T8	Houston Co., GA	Mild	Systemic mosaic	Chlorotic blotches

* Mechanical transmission of cell sap to cotyledons and primary leaves.

Table 4. Shirofugen and ELISA reactions of trees indexed in 1980-81 for Prunus RSV in orchards showing signs of decline.

	Georgia County			
Tree Condition	Houston	Peach	Brooks	Talbot
No symptoms	41/98*	16/102	0/60	0/40
Dieback of terminal twigs only	7/10	3/10	0/60	0/10
Dieback and bark necrosis cankers	19/26	8/21	-	-
Dieback, cankers, bark splitting	38/46	13/35	-	-
Dieback, cankers, splitting, sprouts	5/12	10/35	-	-

* Positive rxs/ trees indexed.

PRUNUS NECROTIC RINGSPOT VIRUS INFECTION AND CANKER 1/

J. G. Barrat, Professor of Plant Pathology, West Virginia University Experiment Farm, and B. E. Otto, Research Technician, USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430

Peach trees in West Virginia orchards are subject to several disorders which shorten their lives. The more serious of these are winter injury, mechanical injury, pine vole girdling, peach tree borer, lesser peach tree borer, Cytospora canker, Prunus stem-pitting, Prunus necrotic ringspot virus (PNRSV) and nematodes. The extent of damage by nematode feeding on roots and virus transmission is not fully known, but may be a serious problem. Cytospora infections following mechanical or winter injury often establish cankers which girdle the limbs of crotches, and kill branches and trunks of trees. These infections are aggravated by the peach tree borer and lesser peach tree borer, which contribute to the enlargement of cankers and the loss of limbs. In addition, pine voles and peach tree borers girdle the trees at the crown and cause severe wilting injury or death. Prunus stem-pitting, caused by tomato ringspot virus, causes tree death or weakens the tree, rendering it more susceptible to low temperature killing. Prunus necrotic ringspot virus infection symptoms must be clarified to aid in disease identification. Cytospora infections add to the difficulty in recognizing PNRSV infection.

The first description of Prunus necrotic ringspot virus on peach was based on bark and foliar symptoms (6). Descriptions in subsequent papers (2, 3, 5, 9) focused on chemical, physical and indexing properties of the virus, rather than on physical description of symptoms on peach for field identification. Cochran et al. (7) and Nyland et al. (16) included some bark symptoms, but concentrated more on foliar symptoms.

The purpose of this paper is to determine the extent of PNRSV infection in peach trees in northeastern West Virginia orchards and to describe certain external symptoms of this infection for field identification.

Materials and Methods

During the past two years, terminal shoots were collected to assay for PNRSV and prune dwarf virus (PDV) by enzyme-linked immunosorbent assay (ELISA). In 1983, 620 peach and nectarine trees were sampled from 23 blocks in 15 orchards. In 1984, 644 samples were taken from 25 blocks in 9 peach orchards. Between 25 and 28 samples were collected from 5 to 7 trees in 4 to 5 adjacent rows in each block. Each sample was taken from a vigorously growing shoot close to the area of major scaffold limb origin. A 10-inch section of terminal shoot and leaf growth was cut and placed in an insulated ice chest and the samples processed within 20 hours of collection.

ELISA indexing was performed following the procedures of Clark and Adams (4). Antisera to sour cherry isolates of PNRSV and PDV were obtained from R. W. Fulton, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53706.

1/ Published with the approval of the Director of the West Virginia Agricultural and Forestry Experiment Station as Scientific Paper No. 1946.

An additional antiserum isolate of PDV was obtained from the American Type Culture Collection (ATCC), Rockville, Maryland 20850.

Samples were held overnight and portions of a few leaves and terminal buds were ground in a Tissuemizer (Tekmar Co., Cincinnati, Ohio 45222) in 1:20 ration (w/v) with PBS-tween PVP buffer. Dormant buds were used where leaf tissue was not available. Enzyme-globulin conjugates were used at 1:500 v/v. Wells of microtiter plates were coated with 200 ul of globulin at 5 ug/ml. Reaction intensity was measured photometrically at 410 mm with a Dynatech Microelisa Mini Reader MR590 (Dynatech Laboratories, Inc., Alexandria, Virginia 22314). Controls included two healthy peach leaves and two diseased peach leaves per 60 sample wells. ELISA reactions giving absorbances equal to or greater than twice the average reading for healthy control samples were regarded as positive (13).

Prunus necrotic ringspot virus infections in peach trees are mostly latent (3, 8, 12, 17). Some foliar symptoms--ringspots, shotholes, distorted leaves (7, 16)--may occur, but in this area, they are elusive and difficult to associate with PNRSV infection in peach. However, bark symptoms in the form of cankers, gumming, fissures, dieback (6) and "bubble bark" pustules (1) do occur and seem to be consistent with PNRSV infection. Various bark conditions were recorded in 1984.

Results

In 1983, 21.6% of the trees sampled gave a positive ELISA response to PNRSV infection in 21 of 23 orchards. Trees ranged from 1 to 22 years of age. When grouped by 4-year age increments, younger trees has less infection than older trees. However, in one collection of 22-year old trees, infection was less than in the 9 to 12 and 5 to 8-year old age groups. One and 2-year old trees averaged 11.25% PNRSV infection with a range of 0 to 16% in six blocks of trees (Table 1). None of the 256 trees indexed for PDV by the ELISA system gave a positive reponse.

In 1984, 33% of the trees sampled were positive to PNRSV infection in all 25 of the orchard blocks. Again, in the different age groups, the frequency of infection increased as age increased. One to 3-year old trees averaged 15.78% PNRSV infection. This infection level represented infection in the trees as they were obtained from the nursery, since they had not blossomed and nematode transmission of PNRSV has not been verified in this country (Table 2). None of the 162 peach trees tested for PDV with the ELISA system gave a positive response.

We were concerned with the lack of any positive response to PDV antisera with ELISA indexing on peaches, so 28 trees in a 20-year old Montmorency sour cherry orchard were sampled further. Of these samples, 89% were positive to PNRSV antiserum and 82% were positive to PDV antiserum with the ELISA system. The Fulton and ATCC PDV antisera gave the same ELISA reactions.

Discussion

Several years ago, in the search for causes of loss of limbs on peach and nectarine trees and the early decline of these orchards, our attention was drawn to cankerous infections incited by Cytospora sp. fungi (11) and their involvement with mechanical injury and winter injury. We assumed these fungal infections were the cause of most of the losses. Early in the investigation, we discovered that stem-pitting virus infections accounted for a considerable loss of younger trees and some older trees. Symptoms of stem-pitting soon became recognized, and control measures were taken (18)

which resulted in a reduction of this disorder in orchards.

However, trees coming into bearing and older trees still suffered the cankerous loss of limbs as well as death of trees. Cytospora canker infections and lesser peach tree borer infestations certainly caused some of these losses, but it was obvious that some other agent was involved. Bud inoculations from infected peach trees into peach trees and greenhouse grown peach seedlings resulted in severe shock reactions--gumming, cankers, fissures, bark peeling, dieback--typical symptoms of PNRSV and PDV infections. We began extensive indexing with the enzyme-linked immunosorbent assay using PNRSV and PDV antisera from Dr. R. W. Fulton and associated many of the declining trees with positive PNRSV reactions.

The descriptions for PNRSV on peach in the literature are somewhat limited, citing only briefly written descriptions or photographs of entire trees which are inadequate to identify the bark symptoms of the disease in the orchard. Prunus necrotic ringspot virus cankers often start on a clear area of the bark on limbs or trunk. Early symptoms are short cracks or fissures on the trunk that resemble growth cracks. Cracks and gumming have not been observed to appear on wood that is less than four years old. One of the first symptoms of PNRSV infection is a vesicle of gum occurring on the bark of the trunk or scaffold limb on 5 to 6-year old wood. The gum arises from a necrotic channel in the phloem extending from the cambial area to the bark and is first emitted from small horizontal cracks which soon change to a small vertical crack and increases in length. Longitudinal fissures and rough bark then may develop on the trunk and lower portions of the scaffold limbs. On six-year old wood, the necrotic tissue formed beneath the bark at gumming sites begin to expand, restricting the flow of sap and causing a slow girdling and death of the terminal portion of the branch. Sucker shoots are often formed below the canker (Figures 1 and 2).

The effects of PNRSV infection begin to appear about the 6th year. Affected limbs have shortened terminal growth, small fruit, develop early leaf yellowing and leaf fall. Affected limbs may be few or include all of the scaffold limbs and appear not to survive low winter temperatures. Some vigorous infected trees, however, live for several years in various stages of symptom development. Often infected trees have dead limbs or have had limbs pruned off by the time they are 8 to 12 years old. This reduces fruit production and contributes to the general poor appearance of the orchard.

An additional symptom, which we associate with PNRSV infection, has been observed for several years on some varieties of peaches and nectarines. We refer to this symptom as "bubble bark", and it is distinquished by round, oblong, or linear pustules of raised or swollen bark tissue which develop on the surface of the scaffold limbs and trunk. Beneath the epidermis of the bark, the swellings are composed of soft parenchymateous tissue which is at first white then dark in color, and raises the bark about 1/16 of an inch (Figure 3).

"Bubble bark" swellings have not been observed on branch growth less than five years old and are not always associated with a gumming site. The "bubble bark" symptom may occur before, after, or simultaneous with gumming and fissure development. Although the symptom is distinctive and is associated with other PNRSV symptoms on the tree, it has not been observed on many older infected trees. Most trees showing "bubble bark" have indexed positive for PNRSV with ELISA. In bud-inoculation transmission attempts, so far, trees have yet to develop this particular symptom. This symptom has been observed on the following varieties: Redhaven, Glohaven, Jerseyland, M. A. Blake, J. H. Hale, Loring, Coronet, Sunhigh, Redskin peaches and Redgold nectarines.

Cytospora sp. fungi invade the weakened tissue caused by the cankers of PNRSV infection. The fungus becomes established, expands, and soon causes an extensive canker up and down the limb in a manner similar to canker development when the organism invades a pruning cut, mechanical or winter injury. Pycnidia are produced and indicate the presence of the fungus. Infected limbs are often killed (Figure 4).

Prunus necrotic ringspot virus infections are latent within the young peach trees as they come from the nursery. The disease is perpetuated in the stock from infected budwood sources, or transmitted to the seedling and scion from infected seed source trees. The rate of seed transmission to seedlings may be expected to be low. Cation (2) and Fridlund (8) suggest 2-4% transmission, which was recently substantiated by Mink and Aichele (15). However, Millikan (14) obtained 15% infection in peach seedlings.

In our indexing of 369 one to three-year old peach trees, we found a range of 0 to 32% PNRSV infection; none with visual symptoms. The occurrence of root sucker growth on some trees may be an indication of infection, but we found this symptom to be inconsistent. Prunus necrotic ringspot virus infections do not appear to hamper the growth of one to four-year old trees.

We found the amount of PNRSV infection in various peach orchards to be variable (Tables 1 and 2). Indexing by ELISA indicated a range in 1983 and 1984 of 0 to 77%, but with an average of 28% in 1983 and 31% in 1984 in trees of all ages. Even in lots of similar trees obtained from the same nursery (Table 2. Redhaven I to V), the range of infection varied from 8 to 32%. We assume that the 1-3-year old trees had produced little or no blossoms and therefore, had not been exposed to virus transmission by pollen. Thus, the PNRSV infection was most likely present in the trees when purchased and resulted from the use of infected budwood. Since a five or six-year lapse of time occurs before PNRSV symptoms develop, it is difficult to trace the presence of these infections to infected nursery stock. However, this is a strong indication that a good portion of the decline in our peach orchards is related to PNRSV infection, and that the virus was present in the trees when planted.

Trees in the age group of 1 to 4 years in both years had a range of 0 to 32% infection with an average in 1983 of 11% and an average of 16% in 1984. This rate of infection is well above the 4% one might normally expect from seed transmission. The virus, therefore, is apparently coming from infected budwood sources. The initial rate of infection is important when one considers the additional possibility of pollen and nematode transmission.

Trees in the age group of 5 to 8 years in both years had a range of 0 to 68% PNRSV infection with an average in 1983 of 14.5% and an average in 1984 of 44%. The percent PNRSV infection in the 1983 five to eight-year old trees is comparable to the 11 to 16% infection in 1 to 4-year old trees, and this infection may be considered to have been present in the nursery stock. The higher rate of 44% PNRSV infection in 1984 five to eight-year old trees may also indicate a higher rate of infection in the nursery stock, or it may indicate PNRSV transmission by pollen, nematodes or some other agent.

In the age group of 9 to 14 years, 14 to 77% of the trees were infected with PNRSV and averaged 48% in 1983 and 38.5% in 1984 as detected by ELISA.

In the 22-year old orchard, the average infection in 1983 was 28% and in 1984 48%. At this age, one might expect a much higher rate of PNRSV infection if pollen transmission had taken place. In the sour cherry orchard, PNRSV infection was recorded in

89% of the trees. Widespread pollen transmission is known in sour cherries (10).

Some of the younger peach blocks will be indexed periodically to determine the rate of spread of PNRSV infection, possibly by pollen or nematode transmission or by other means. At present, We have no indication of pollen transmission in peaches, and we have no record of nematode or insect transmission of PNRSV in this country. This will require further investigation.

Table 1. Prunus necrotic ringspot virus infection in peach trees as indicated by ELISA - 1983.

	1983 Date		Variety	Age Group	Number Indexed	Number PNRSV Positive		Percent Positive
1.	8/31	D*	Sunhigh	1	27	3		11.11
2.	9/8	G	Various	2	27	0		0.0
3.	8/23	J	Various	2	27	3		11.11
4.	8/16	A	Loring	2	27	4		14.81
5.	8/23	C	Redhaven	2	27	4		14.81
6.	6/29	C	Redhaven	2	25	4		16.00
			Total		160	18	Avg.	11.25
7.	9/8	K	Redhaven	5	27	5		18.51
8.	8/16	L	Redskin	6	27	0		0.0
9.	7/20	M	Redskin	6	27	1		3.70
10.	7/20	M	Glohaven	6	27	2		7.40
11.	7/13	O	Redhaven	7	27	6		22.22
12.	7/26	P	Redh., Lor., Redsk.	7	27	1		3.70
13.	7/26	C	Glohaven	8	27	8		29.62
14.	8/3	N	Glohaven	8	27	8		29.62
15.	8/9	Q	Blake, Loring	8	27	2		7.40
16.	8/9	R	Redhaven, Loring	8	27	4		14.81
17.	9/20	K	Various	8	27	6		22.22
			Total		297	43	Avg.	14.47
18.	3/1	S	Redhaven	9	28	12		42.85
19.	7/6	T	Redgold Nectarine	9	26	8		30.76
20.	7/13	J	Redhaven	9	27	14		51.85
21.	2/24	S	J. H. Hale	10	29	18		62.06
22.	7/6	S	J. H. Hale	10	28	14		50.00
			Total		138	66	Avg.	47.82
23.	6/29	C	Loring	22	25	7		28.00
			Total (All)		620	134	Avg.	21.60

*Capital letters indicate different orchards.

Table 2. *Prunus* necrotic ringspot virus infection in peach trees as indicated by ELISA - 1984.

	1984 Date		Variety	Age Group	Number Indexed	Number PNRSV Positive		Percent Positive
1.	8/14	A*	Rio Oso Gem	1	28	3		10.7
2.	9/5	B	Redskin	3	28	4		14.2
3.	9/21	C	Redhaven I	3	25	6		24.0
4.	2/23	C	Redhaven II	3	25	5		20.0
5.	2/27	C	Redhaven III	3	25	8		32.0
6.	3/6	C	Redhaven IV	3	28	2		8.0
7.	3/12	C	Redhaven V	3	25	3		12.0
8.	4/18	D	Loring	3	28	2		7.1
			Total		209	33	Avg.	15.78
9.	7/11	E	Various	5	28	15		53.5
10.	10/3	C	Redskin	6	12	6		50.0
11.	9/10	F	Blake	6	28	5		17.8
12.	10/3	G	Loring, Sunhigh	7	16	7		43.7
13.	7/23	H	Loring	7	28	7		25.0
14.	7/31	H	Loring	7	28	6		21.4
15.	10/10	C	Redskin	7	28	19		67.8
16.	10/17	C	Redskin	7	28	17		60.7
17.	8/7	D	Glohaven N	8	24	9		37.5
18.	8/21	D	Glohaven S	8	28	19		67.8
			Total		248	110	Avg.	44.35
19.	4/9	I	Harken	10	27	4		14.8
20.	1/9	G	Glohaven	11	26	15		57.6
21.	4/25	G	Blake	12	28	7		25.0
22.	5/8	F	Loring	12	28	6		21.4
23.	1/26	F	Redhaven	14	26	20		76.9
			Total		135	52	Avg.	38.5
24.	3/20	C	Loring 127-31	22	26	10		38.4
25.	4/3	C	Loring 130-34	22	26	15		57.6
			Total		52	25	Avg.	48.07
			Total (All)		644	220	Avg.	34.16

*Capital letters indicate different orchards.

Literature Cited

1. Barrat, J. G. 1984. Prunus necrotic ringspot--another virus problem in West Virginia peach orchards. The Mountaineer Grower (Martinsburg, WV 25430), No. 458, June. pp 22-28.

2. Cation, D. 1961. A determination of necrotic-ring-spot virus in Michigan peach orchards and nursery stock. Plant Disease Reptr. 45: 109-111.

3. Civerolo, E. L. and S. M. Mircetich. 1972. A peach isolate of Prunus necrotic ringspot virus. Phytopathology 62: 529-532.

4. Clark, M. F. and A. N. Adams. 1976. Laboratory notes on the ELISA technique. East Malling Res. Stn. Maidstone, Kent. 6 pp.

5. Cochran, L. C. 1950. Passage of the ring-spot virus through peach seeds. (Abstr.) Phytopathology 40: 964.

6. Cochran, L. C. and L. M. Hutchins. 1941. A severe ringspot virosis on peach. (Abstr.) Phytopathology 31: 860.

7. Cochran, L. C., L. M. Hutchins, J. A. Milbrath, G. L. Stout and S. M. Zeller. 1951. Ring Spot. In Virus Diseases and Other Disorders With Viruslike Symptoms of Stone Fruits in North America. U. S. Dept. Agr., Agr. Handb. 10, pp. 71-80.

8. Fridlund, P. R. 1966. Transmission of latent viruses in commercial peach seed. Plant Disease Reptr. 50: 740.

9. Fulton, R. W. 1970. Prunus necrotic ringspot virus. Commonw. Mycol. Inst./ Assoc. Appl. Biol. Descriptions of Plant Viruses No. 5. June. Kew, Surrey, England.

10. George, J. A. and J. R. Davidson. 1963. Pollen transmission of necrotic ring spot and sour cherry yellows viruses from tree to tree. Can. J. Pl. Sci. 43: 276-288.

11. Jones, A. L. and T. B. Sutton. 1984. Perennial canker. In Diseases of Tree Fruits. Coop. Ext. Ser. Mich. State Univ. pp. 46-47.

12. Lazar, A. C. and P. R. Fridlund. 1967. The incidence of laten viruses in Washington peach orchards. Plant Disease Reptr. 51 (12) 1063-1065.

13. Lister, R. M., W. R. Allen, D. Gonsalves, A. R. Gotlieb, C. A. Powell and R. F. Stouffer. 1980. Detection of tomato ringspot virus in apple and peach by ELISA. Acta Phytopathol. Acad. Sci. Hung. 15 (1-4): 47-55.

14. Millikan, D. F. 1959. The incidence of the ring spot virus in peach nursery and orchard trees. Plant Disease Reptr. 43: 82-84.

15. Mink, G. I. and M. D. Aichele. 1984. Detection of Prunus necrotic ringspot and prune dwarf viruses in Prunus seed and seedlings by enzyme-linked immunosorbent assay. Plant Disease 68: 378-381.

16. Nyland, G., R. M. Gilmer and J. D. Moore. 1976. "Prunus" ring spot group. In Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. U. S. Dept. Agr., Agr. Handb. No. 437, pp. 104-132.

17. Pine, T. S. 1964. Influence of necrotic ringspot virus on growth and yield of peach trees. Phytopathology 54: 604-605.

18. Stouffer, R. F., D. M. Soulen and S. H. Smith. 1975. Spread and control of Prunus stem pitting. ACTA Hortic. 44: 107-112.

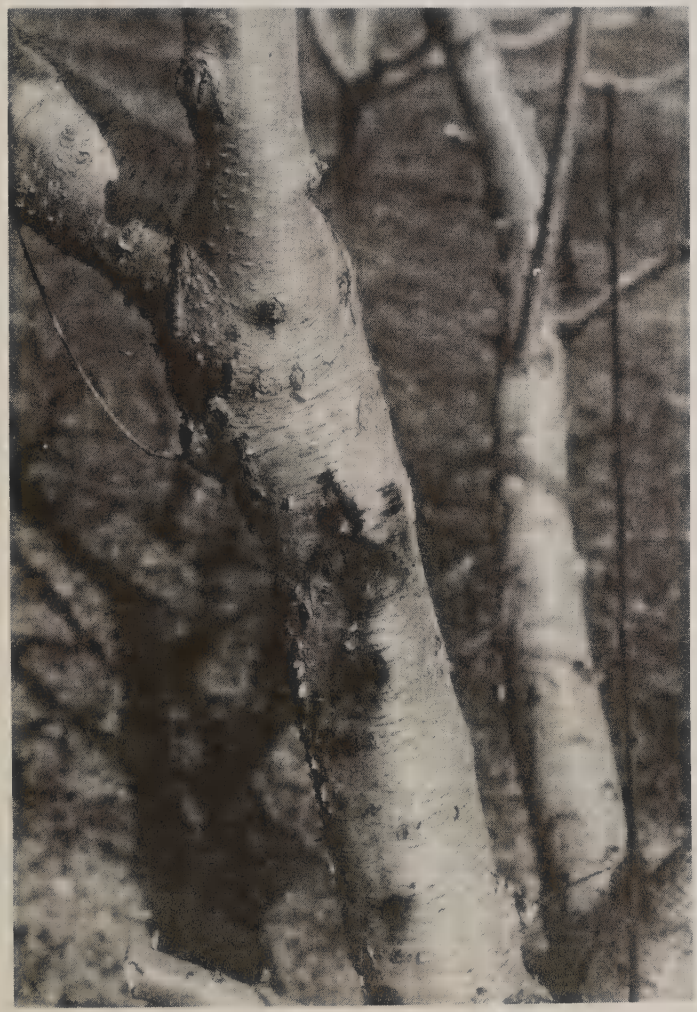


Figure 1. PNRSV fissures on peach scaffold limbs.

Figure 2. PNRSV fissures on peach trunk.

Figure 3. PNRSV "bubble bark" on peach trunk and scaffold limbs.

Figure 4. Cytospora and lesser peach tree borer canker.

QUANTIFICATION OF MECHANICAL DAMAGE TO RED TART CHERRY TREES AND ITS POTENTIAL INFLUENCE ON TREE DECLINE: A PROGRESS REPORT

B. F. Cargill, Gary VanEe, G. K. Brown, Richard Ledebuhr and Keith Price
Agricultural Engineering Department
Michigan State University
East Lansing, Michigan 48824

Introduction

Various harvest mechanization components may influence cherry tree decline. In the spring of 1983 a block of approximately 1000 young Montmorency cherry trees was mapped out of an orchard in Oceana County, Michigan. The orchard had been uniformly harvested the previous year (1982) with a one-man Friday harvester using a Friday tri-clamp and Friday pads. All of the mechanical damage from this first year of machine harvest (1982) was recorded for each tree. The mechanical damage was to be recorded for each of the 1000 trees for a minimum of five years for the purpose of observing a potential tree damage correlation between various mechanical harvesting components and cherry tree decline.

Purpose

The purpose of establishing this uniform block of trees (10 rows and 100 trees per row) was to verify and quantify laboratory research results and model analysis under long range actual commercial orchard management.

Harvester clamp designs, pad designs, and pad pressure (another project) are being analyzed and modeled under laboratory conditions. The results of this research needs to be incorporated, where practical, into the commercial test site.

Factors to be observed over the long duration in the commercial site were envisioned to include:

- Type of harvester
- Harvester clamp system
- Pad systems from various manufacturers and experimental pad systems
- Pad pressures (system pressures and variations in pressures applied to the tree trunk)
- Shake duration (seconds)
- Type of shake (continuous or intermittent)
- Time of harvest (a.m. - p.m.)

The Orchard

The Montmorency (Mahaleb root stock) trees were purchased from the Hawley Nursery, Hart, Michigan. The orchard was planted in 1977 as a replant of a thirty-five year old orchard. The site is an excellent cherry orchard site with rolling land and air drainage to the southwest. The site is located two miles north of Hart on the west side of Oceana Drive, Oceana County, Michigan. The 1000 tree site is part of a twenty acre block all replanted in 1977. The rows are orientated east-west (not the typical north-south orientation) due to the rectangular shape of the twenty acre block.

The block of 1000 trees used in this study is located along the entire south side of the orchard site. The block has ten rows (18 foot spacing between rows) with approximately 100 trees per row (12 foot spacing within the row). There are two rows of cherry trees to the south of the recorded block that are considered "guard" rows.

The twenty acre block is part of a sixty acre commercial red tart cherry orchard managed by the Vernon Bull Orchards, Inc.

The Records

For recording purposes the ten rows of trees in this block are assigned letters (AAA, AA, A, B, C, D, E, F, G, H) and each tree is numbered with a metal tag for identification within each row (#0-100). See orchard site layout, Fig. 1.

Figure 2 is an example of the recording sheet used in this study. The "damage" observed in the orchard will fall into the six categories with damage location also being noted by using the directions of the compass and the height of the damage from ground level. Damage is also noted to be fresh, hd=healed, or hg-healing.

The damage is defined as follows:

1. Barked: Damage done only to the bark of the tree. No visible damage to the cambium.

2. Crushed: Noticeable flattening due to excessive clamping force by the harvester.

3. Gum: Active gum on the tree.

4. Limb Scar: Scars left on the tree due to pruning cuts.

5. Scar: Damage that extends into or beyond the cambium

6. Lean (to): Which direction the tree leans (if any).

7. Other: May include non-mechanical type damage such as deer, rabbit, and mouse damage, holes dug at the trunk by animals, etc.

Trunk diameters are taken to plot the growth of the trees. The data sheet indicates as to whether the trunk is straight or crooked.

Individual tree evaluations are recorded three times a year: 1) before harvest, 2) during harvest, and 3) in early fall. See Fig. 2 for an example of the record sheet.

Color photos (4"x5") have been taken of any unusual occurrence to a tree. The data sheet indicates when a picture is taken. The combination of the data sheets and color photographs provide an excellent periodic record of events influencing a given tree.

The Harvester

It was planned to have the majority of the basic harvesting done with a Friday, one-man, tri-clamp harvester. This harvester was used for the initial harvest in 1982. In 1983 an over-the-row harvester designed and built by Don Peterson,

USDA-ARS, Kearneysville, West Virginia, was used on some of the test rows (See Table 1).

During this study the effect of different design parameters, such as clamping force, shake duration, peak pad pressures, pads, clamps, time of harvest, etc. were investigated with respect to their influence on damage to the cherry trees. Table 1 shows the different types of mechanical treatments that each row has undergone.

The bark on one row of trees (Row AAA) was vertically scored with a razor blade. The bark was vertically scored from 3 to 18" from the ground. The blade was controlled to score to a depth of one-half the cambium. Trees were scored with three and six vertical scores around the periphery. The scoring was done in the fall of 1983 and the spring of 1984. See Table 2 for a detailed description of the scoring.

Results and Discussion

1984 is only the second year of this five year study, therefore, no results can be discussed. However, there appears to be some trends developing.

One of these trends is that all of the mechanical harvesting damage caused by the trunk shaker is not visible or noticeable during harvest. A considerable amount of the damage to the trunk is observed later in the season (fall).

Another trend observed during harvesting in 1984 was that harvester damage seems to occur more readily in the early morning hours than in the afternoon or evening.

It is also important to notice that after only three years of mechanical harvest, 28% of trees have no visible mechanical damage, 67% of the trees have visible damage, and 5% have severe visible damage[1].

[1] Percentages do not include Row AAA.

Table 1. Mechanical Treatments for the Hart Cherry Tree Decline Study for 1983 and 1984.

	Row AA		Row A		Row B	
	1983	1984	1983	1984	1983	1984
Harvester	Friday	Friday	USDA	Friday	USDA	Friday
Clamp	Tri	Tri	C	Tri	C	Tri
Pads	Friday	Friday	Kilby	Kilby	Kilby	Kilby
Peak psi	1200	1200	250-350	150-350	250-350	150-350
Shake, sec	10	10	3	4	9	10
Time		a.m.		Noon		a.m.

	Row C		Row D		Row E	
	1983	1984	1983	1984	1983	1984
Harvester	USDA	Friday	USDA	Friday	USDA	Friday
Clamp	C	Tri	C	Tri	C	Tri
Pads	Friday	Friday	Kilby	Kilby	Kilby	Kilby
Peak psi	400-500	1000	250-350	150-350	250-350	150-300
Shake, sec.	3	10	3	4	9	10
Time		p.m.		Noon		a.m.

	Row F		Row G		Row H	
	1983	1984	1983	1984	1983	1984
Harvester	USDA	Friday	Friday	Friday	Friday	Friday
Clamp	C	Tri	Tri	Tri	Tri	Tri
Pads	Friday	Friday	Friday	Friday	Friday	Friday
Peak psi	400-500	1000		1200	1200	1000-2000
Shake, sec.	3	10	10	10	10	10
Time		p.m.		p.m.		a.m.

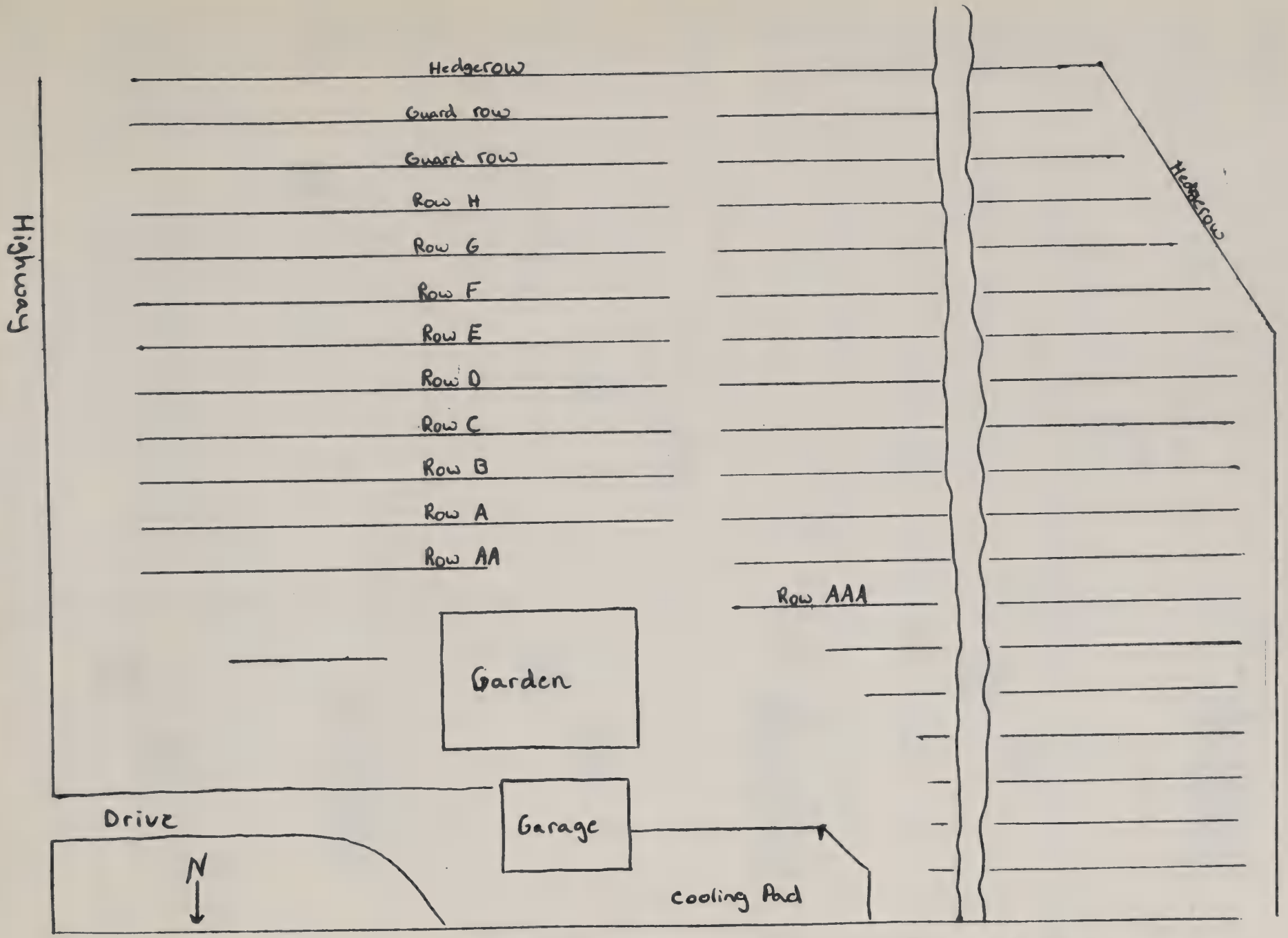


Fig. 1. Twenty Acre Orchard Block and the Orchard Test Site Layout (Plot Plan of Rows and Trees).

ROW J NUMBER 98 9(TREE NUMBER)

	N	NW	W	SW	S	SE	E	NE
BARKED	■	■	■	■				
CRUSHED								
GUM								
LIMB SCAR								
SCAR			■					
LEAN (TO)					■			
OTHER								

Date 7-23-84

Trunk diameter (18") 4.5"

~~Straight trunk~~

Crooked trunk

Bad crotch

Picture: Yes/No

Scar and barking are damage from last year's harvesting. Healing well.

	N	NW	W	SW	S	SE	E	NE
BARKED	■	■	■	■				
CRUSHED								
GUM								
LIMB SCAR								
SCAR			■					
LEAN (TO)					■			
OTHER								

Date 7-24-84

Trunk diameter (18") 4.5

[redacted]ght trunk

Crooked trunk

Bad crotch

Picture: [redacted]/No

Harvester redamaged tree over lasts year damage.

	N	NW	W	SW	S	SE	E	NE
BARKED	■	■	■	■				
CRUSHED								
GUM								
LIMB SCAR								
SCAR			■					
LEAN (TO)					■			
OTHER								

Date 10-19-84

Trunk diameter (18") 4.75

Straight trunk

Crooked trunk

Bad crotch

Picture: Yes/No

Harvester damage healing well.

Fig. 2. Data Sheet

TROPHOTYPIC DIFFERENCES AMONG CRICONEMELLA XENOPLAX POPULATIONS ATTACKING PRUNUS PERSICA

Rich Owings
Department of Horticulture, Clemson University
Clemson, SC 29631

The ring nematode, Criconemella xenoplax, has been shown to be a primary contributing factor in initiating the PTSL complex (Nyczepir, et al, 1983). Although Criconemella xenoplax can be found in virgin soils, the damage associated with PTSL does not usually occur on such sites. It has been suggested (as an explanation of these site differences) that the previous host influences the ability of the nematode to reproduce on and/or attack subsequent hosts (Mojtahedi and Lownsbery, 1975). Barker (1982) states that "...large populations of C. xenoplax occur in soils supporting healthy and short life trees (C. M. Clayton, unpublished)." Barker and Clayton (1973) suggest the possible existence of various trophotypes among C. xenoplax populations.

A trophotype is defined as a specific population which has the ability/disability to attack and/or reproduce on a specific host to the extent that the population differs from other populations in this ability. This research is designed to test this hypothesis and to determine if trophotypes exist.

Methodology

Soil samples were taken from six PTSL sites, and from five non-peach sites, at various locations in South Carolina and Georgia. The PTSL sites all involved orchards which had been planted in peaches for more than one generation, and were showing moderate to severe short life damage. The non-peach or virgin sites were areas where the researcher could be reasonably certain that peaches had never been planted. The five non-peach sites included three pecan orchards, a raspberry planting, and an old field location.

Four of these populations are of special interest because they represent PTSL sites and non-peach sites in close proximity to each other, and therefore are an excellent test for the existence of trophotypes (i.e. - all factors are held constant except for the source host). Populations 1 and 7 are from the USDA Southeastern Fruit and Tree Nut Research Laboratory in Byron, Georgia, and are separated only by a distance of several meters (being located on opposite sides of a dirt road). Populations 5 and 8 in Johnston, South Carolina are separated by a distance of approximately one hundred meters. In both situations one site is a peach orchard and the virgin site is in pecans.

Samples of the eleven populations were sent to Dr. A. Morgan Golden, USDA Nematologist, for species verification. All populations were determined to consist exclusively of C. xenoplax except for populations 10 and 11. Population 10 contained mainly Nothocriconemella mutabilis and a few specimens of Criconemella sphaerocephala. Population 11 contained a previously unknown species, Paracriconema n. sp. and a couple of specimens of Criconemella sp. (Golden, personal communication; Ebsary, 1981).

The populations were tested in two separate experiments. Experiment A was inoculated two weeks prior to experiment B. Each experiment was performed in a

separate greenhouse section. Nemaguard seedlings were inoculated with approximately 250 ring nematodes per container.

Populations were sampled every twenty eight days, starting two months after inoculation, with the fifth and final sample taken 178 days after inoculation. One fifth of all the plants in the experiment were removed for sampling at each of the five dates.

Experiment A contained populations 1, 2, 3, 4, 5, 6, 7, 8, 9, and 12. Experiment B contained populations 1 through 12. Population number 12 represents a non-inoculated control.

In experiment B populations 8, 9, 10, and 11 are not represented in all dates due to the lack of initial population increase. Because of this lack of inoculum for some populations the initial extraction dates were dropped in favor of retaining later dates.

At inoculation (day 0) height measurements were taken on all plants. At each sampling date, height measurements were taken for all remaining plants, while the plants to be sampled on that date were cut at the soil line, and their root systems separated from the soil in the container. The shoot and root samples were dried at 65°C for seven days. Shoot and root samples were weighed immediately upon removal from the drying oven.

Nematodes were extracted from the soil within 24 hours of removing the plants. A semi-automatic elutriator was used following the Jenkins (1964) method. Five hundred cc of soil was used and one fifth of the elutriate water was collected, resulting in the collection of nematodes from 100 cc of soil. All nematodes were counted and identified within 48 hours of elutriation using a 30X stereoscope.

Results and Discussion

As mentioned earlier, the two experiments were conducted in separate greenhouse sections. Six days prior to the extractions due for date 3, the cooling system in the greenhouse containing experiment A shut down for several days. During this time the soil in the pots had reached temperatures as high as 41°C (106°F). Soil temperatures of 32.2°C (90°F) are reportedly high enough to kill C. xenoplax (Zehr, personal communication). The populations survived this short period of high temperatures, although it did affect their reproductive behavior. Most of the figures for experiment A show a population decline following the second extraction date. While not planned, this did provide information concerning how various populations of C. xenoplax react differently to heat stress. This decline and the subsequent population recovery represents the major difference between experiments A and B.

Very few plant measurements were significant. In experiment A only six significantly different measurements occurred, though twenty occurred in experiment B. This difference between experiments is probably due to the cooling system malfunction in experiment A.

The populations (1-9) containing C. xenoplax multiplied at varying rates. In both experiments PTSL source populations (1-6) appeared to increase at a slightly faster rate when compared to virgin site populations (7-9), although significant differences only occurred at dates 1 and 4 in experiment A and at date 2 in

experiment B. Populations 1, 2, 3, 4, and 8 were fairly consistent in being the strongest populations in both experiments. All of these except population 8 originated in peach orchards.

Among PTSL populations it appears that populations 1, 2, 3, and 4 increased at a more rapid rate than the others in both experiments. Significant differences occurred among PTSL populations at all dates except date 5 in experiment A and date 4 in experiment B. Population 6 seems to be least vigorous of the PTSL group. Population 5 also seemed to lag behind other PTSL populations, especially in experiment B. It is interesting to note that populations 2 and 6 were the only PTSL populations not to decline (between dates 2 and 3) following the cooling system failure, though they both declined at date 3, indicating a possible short term tolerance to heat stress.

Among virgin source populations, groups 8 and 9 were obviously stronger than population 7. Significant differences occurred at dates 2, 3, and 4 in experiment A, and at all possible dates (3, 4, and 5) in experiment B. Population 8 was the only group in Experiment A that did not undergo a decline at any point in the experiment, though their rate of increase slowed following the cooling system failure. This may be indicative that the population is somewhat heat tolerant.

It was previously noted that populations 8, 9, 10, and 11 were not included in all dates in experiment B. A primary concern here was that these populations might be exhibiting trophotypic behavior in being slow to increase.

Populations 1 and 7, and populations 5 and 8 represent the two pairs of adjacent populations previously mentioned as a good test of trophotypes. Looking at populations 1 and 7, we can see that population 1 attained greater levels than the neighboring group (population 7) which came from an adjacent pecan grove. Significant differences ($P>.05$) among nematode counts occurred at all dates in experiment A, and at all except date 1 in experiment B.

When populations 5 and 8 are examined, we see that the virgin population (number 8) tended to exhibit higher levels. Significant differences were noted at dates 2, 3, and 4 in experiment A, but no significant differences occurred between these two populations in experiment B. It has already been noted that population 8 was the only one not to undergo any sort of decline following the cooling system failure in experiment A. Perhaps population number 8 becomes a stronger competitor (relative to other populations) in the presence of heat stress.

Because of the limited amount of inoculum available, populations 10 (*Nothocriconemella mutabilis*) and 11 (*Paracriconema* n. sp.) were used only in experiment B at date 5. No significant differences were found between these two populations, either in nematode counts, or in plant measurements. Neither were plant growth differences found when each species was compared to the control. Highly significant ($P = .0001$) differences in nematode counts did occur when all *C. xenoplax* populations (1-9) were bulked together and contrasted with each of these two populations.

By date 5 the least square means for the log (nematode count + 1) of the nematode populations ranged from a low of 6.07 (for population 6) to a high of 8.10 (for population 4) among *C. xenoplax* populations in experiment B. Population 10 showed a least square means of 1.86, while population 11 was 1.09. Initial inoculation levels of 1.04 (10/100 cc) shows that population 11 increased only slightly over the 178 day period. Peach appears to be, at best, a poor host for these two species.

LITERATURE CITED

1. Barker, K.R., and C.N. Clayton. 1973. Nematodes attacking cultivars of peach in North Carolina. J. of Nemat. 5:265-271.
2. Barker, K.R. 1982. Criconemella in the Southeastern United States. In Nematology in the Southern United States, Southern Cooperative Series Bulletin 276:150-156.
3. Ebsary, B.A. 1981. Generic Revision of Criconematidae (Nematoda): Nothocriconema and related genera with proposals for Nothocriconemella n. gen. and Paracriconema n. gen. Can. J. Zool. 59:1227-1236.
4. Jenkins, W.R. 1974. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 58:76-79.
5. Mojtahedi, H., and B.F. Lownsbery. 1975. Pathogenicity of Criconemoides xenoplax to prune and plum rootstocks. J. of Nemat. 7:114-119.
6. Nyczepir, A.P., E.I. Zehr, S.A. Lewis, and D.C. Harshman. 1983. Short life of peach trees induced by Criconemella xenoplax. Plant Dis. 67:507-508.

PRELIMINARY RESULTS OF FIELD SCREENING PEACHES FOR RESISTANCE TO *CRICONEMELLA XENOPLAX*

David W. Cain[1], W.R. Okie[2], E.I. Zehr[3], and A.P. Nyczepir[4]

Peach tree short life (PTSL) is a serious problem in the southeastern U.S. It is a complex disease in which the ultimate cause of tree death appears to be bacterial canker and/or cold injury (4). Ring nematodes *Criconemella xenoplax* (Raski) Luc & Raski have been implicated as a causative factor in inducing PTSL (2, 3, 4, 5). While Lovell rootstock survives better than other rootstocks (1, 3), no high level of resistance to ring nematodes is known. A source of resistance to ring nematodes needs to be located in order to determine if resistance to the nematode also imparts resistance to the entire PTSL complex.

In 1982, personnel at Clemson and the USDA Tree Fruit and Nut Lab in Byron, GA, initiated a joint project to try to locate a source of resistance to ring nematode in *Prunus* germplasm. Open pollinated seeds were collected from 142 diverse *Prunus* clones. Germplasm included named rootstocks, plant introductions, long lived trees found growing on PTSL sites, feral peaches found in the Appalachian mountains, and several (*Prunus*) species. Six clones propagated from rooted cuttings were also included.

The seeds were planted in a fumigated nursery bed at Clemson, SC, in November 1981. The seedlings were dug in January 1983 and planted in replicated plots at Byron, GA and Elgin, SC. The SC site is a lakeland fine sand and has had a history of PTSL in previous orchards. The surviving 139 lines were planted in 6 replicates of eight trees each in a randomized block design. Some lines had fewer replicates because of limited tree numbers. Trees were spaced 2 ft x 16 ft. Plots were maintained as herbicide stripes with sod middles. Irrigation was applied during dry periods during the summer.

The Byron plots consist of eight replicates of six trees each. Four replicates were planted on a severe PTSL site. The previous orchard was removed during the summer of 1982. The other reps were planted on an oak root rot site. The Byron planting included 5 lines not in the Sandhill planting and omitted 27 lines in the Sandhill planting. Thus, there are 112 lines common to both sites.

In November, 1983 all plots at both sites were sampled for nematodes. In SC, soil samples were removed from both sides of the center six trees of each plot. The soil was well mixed and the sample was held at 3°C until elutriated. A 500 cc sample was extracted using a semiautomatic elutriator. One fifth of the sample was collected. Sugar floatation was used to separate the nematodes from debris. Nematodes were then counted under a binocular microscope. At Byron, the nematodes were hand screened and then separated by sugar floatation.

In addition to ring nematodes, in South Carolina, dagger and spiral nematodes were also counted. At both locations, tree death counts are being made and cause of each death is recorded.

[1,3]Departments of Horticulture and Plant Pathology and Physiology, Clemson University, Clemson, SC, respectively.

[2,4]Research Horticulturist and Plant Pathologist, USDA-ARS, Byron, GA, respectively.

After the first growing season no deaths due to PTSL occurred in S.C. In Georgia, 15 trees died from PTSL and 28 died from oak root rot. Nematode data for both sites were analyzed at Byron via analysis of variance. Both the actual counts and the log of the counts were analyzed. Duncan's multiple range test was used to separate means. Results reveal that nematode counts show extreme variability both between replicates within a site and between sites.

Significant block differences were found for all nematode genera in the Sandhill plots (Table 1). While we have not fully analyzed the data, no apparent relationships exist between population levels of the three nematode genera. Even though blocking significantly reduced random error, variability within lines was so large that line effects were nonsignificant.

At Byron, significant row effects were found. Rows planted in the rows of the previous planting had higher counts than rows planted in former drive middles.

Table 2 further illustrates the amount of variability encountered. Using log transformed counts adjusted for site, block and row effect, the five highest and lowest counts were arbitrarily chosen for illustration since space precludes listing all counts. None of the 10 highest or lowest values of the two sites are common to each other. Only 5 of the 10 combined means are common to one of the other sites.

Three clones were represented two or more times in the experiment because we want to investigate the effect of scion rootstock combinations and seed vs. rooted cuttings. Ring nematode counts for these rootstocks are given in Table 2. The mean counts for Lovell range from 60 to 194; a three fold difference.

In conclusion, screening for resistance to ectoparasitic nematodes is an important goal in Prunus rootstock breeding. It is also a very difficult and expensive task. Even though the present field screening experiment is well replicated (10 reps with a total of 96 trees per line), it is difficult to detect even very large differences. A single growing season is apparently not long enough to allow adjustment of previous nematode levels. We plan to continue monitoring nematode levels to determine long term changes and to follow tree death. While field screening may still allow us to locate a source of resistance to ring nematodes, it is too expensive and slow to be useful in screening seedlings when actual breeding begins. At both Byron and Clemson, greenhouse screening of additional lines has begun. Random variability is also expected to be great but hopefully will be less than in the field. Greenhouse screening is also expensive due to heating and cooling costs and only limited space is available. New innovative procedures are needed before rapid progress can be expected. At Clemson we are investigating the possibility of in vitro screening using tissue cultured plants and axenic nematode cultures.

Literature Cited

1. Dozier, W.A., Jr., J.W. Knowles, C.C. Carlton, R.C. Rom, E.H. Arrington, E.J. Wehunt, U.L. Yadava, S.L. Daud, D.F. Ritchie, C.N. Clayton, E.I. Zehr, C.E. Gambrell, J.A. Brittain and D.W. Lockwood. 1984. Survival, growth and yield of peach trees as affected by rootstocks. HortScience 19:26-30.
2. Lownsbery, B.F., H. English, G.R. Noel, and F.J. Schick. 1977. Influence of Nemaguard and Lovell rootstocks and Macroposthonia xenoplax on bacterial canker of peach. J. Nematol. 9:221-224.
3. Nyczepir, A.P., E.I. Zehr, S.A. Lewis and D.C. Harshman. 1983. Short life of peach trees induced by Criconemella xenoplax. Plant Disease 67:507-508.
4. Weaver, D.J., E.J. Wehunt, and W.M. Dowler. 1974. Association of tree site, Pseudomonas syringae, criconemoides xenoplax, and pruning date with short life of peach trees in Georgia. Plant Disease Reporter 58:76-79.
5. Zehr, E.O., R. Walker Miller and Fred H. Smith. 1976. Soil fumigation and peach rootstocks for protection against peach tree short life. Phytopathology 66:689-694.

Table 1. Block Means for Sandhill Nematode Counts

Rep	Count			Rep	Count		
	Cr	Xi	Sc		Cr	Xi	Sc
3	191	3.4	28	6	169	26	1.0
2	127	5.1	13	5	165	25	10.7
1	54	0.9	4	4	125	21	1.6

Cr - Criconemella, Xi - xiphinema, Sc - Scutellonema

Table 2. Five highest and lowest mean log nematode counts

Location	Sandhill		Byron		Combined	
	Line	Count	Line	Count	Line	Count
	148	1.37	152	0.69	148	1.35
	35	1.47	150	0.69	152	1.39
	143	1.49	78	1.00	150	1.40
	104*	1.50	149	1.05	145	1.49
	139	1.51	77	1.07	78	1.53
	126	2.20	94*	2.20	4*	2.16
	27	2.30	83	2.27	67*	2.19
	88*	2.33	68	2.32	126	2.19
	15	2.48	107	2.42	27	2.28
	91	2.52	110	3.32	91*	2.44

*Resistant to meloidogyne spp.

Table 3. Ring nematode counts on three rootstocks

Cultivar	Clone No.	Source[z]	Sandhill	Byron
Lovell	48	S	194	1.336
Lovell	137	C	123	1.454
Lovell	147	S	66	1.149
Lovell	143	C	60	1.674
Halford	136	C	102	1.706
Halford	45	S	65	1.362
Nemaguard	50	S	152	1.803
Nemaguard	138	C	146	1.417
Nemaguard	141	S	-	1.595

[z]S = seed, C = cuttings
[y]Log of counts

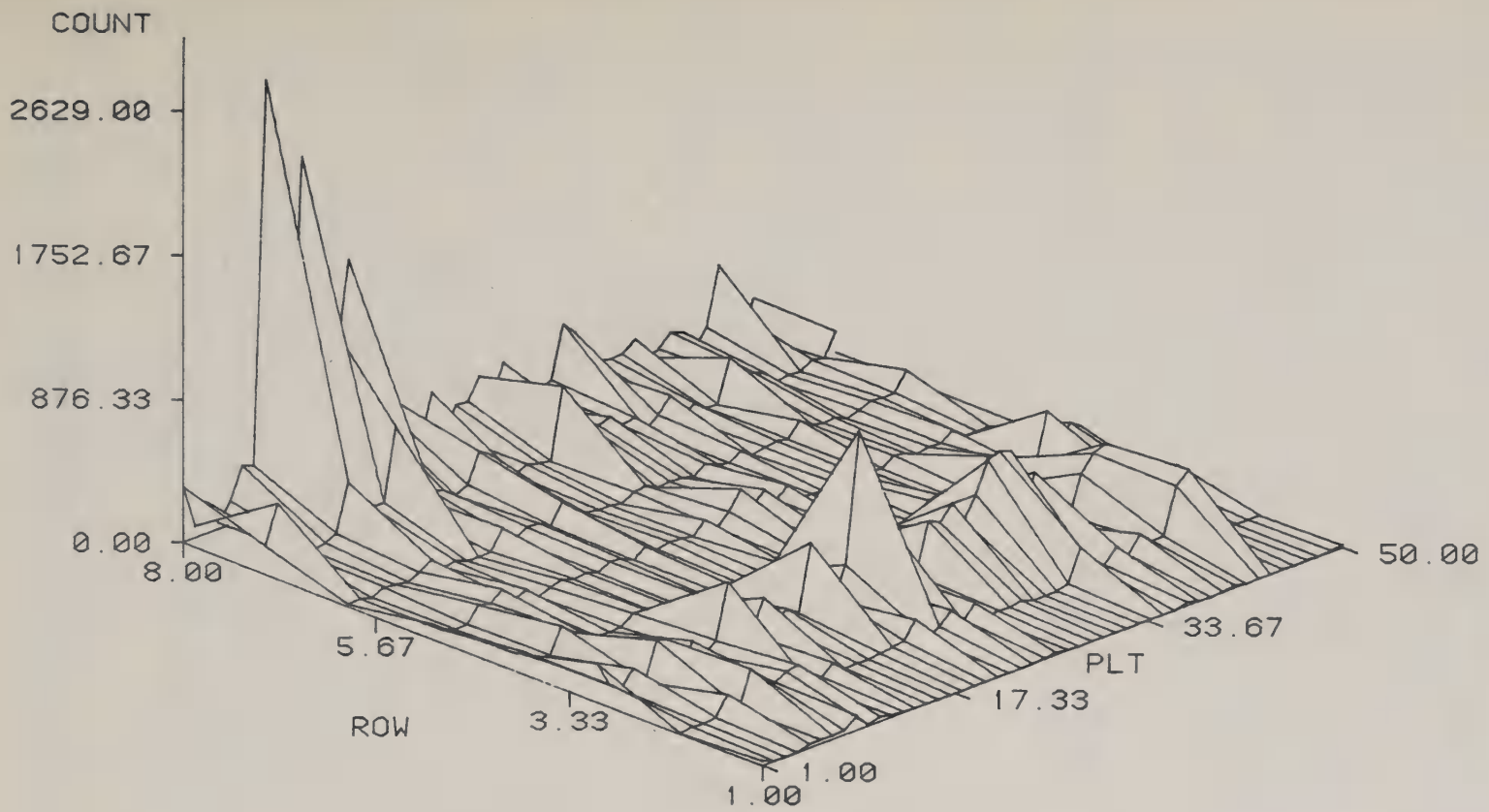


Figure 1. Effect of previous peach orchard tree rows on Byron ring nematode counts.

PHYSIOLOGICAL RESPONSES OF PEACH TREES IN RELATION TO FACTORS OF THE PEACH TREE SHORT LIFE COMPLEX

C. C. Reilly, W. R. Okie, A. P. Nyczepir, and R. R. Sharpe
USDA/ARS, S. E. Fruit & Tree Nut Research Laboratory
Byron, GA 31008

Summary

Peach tree short life in the southeastern United States is associated with old peach land, ring nematode (Criconemella xenoplax), rootstock, fall pruning, and fluctuating winter temperatures. The ultimate cause of tree death appears to be cold injury. Trees on Lovell rootstock generally outlive those on Nemaguard on short-life sites, but little is known of the physiological basis for this superior survival. Efforts to develop long-lived rootstocks are hampered by lack of criteria on which to select superior lines. Criconemella xenoplax infestation on Lovell and Nemaguard seedlings in the greenhouse caused reduced fresh and dry root weight and dry matter contents. Levels of ninhydrin-positive compounds were reduced in stem and roots. Root systems of infested plants had reduced total amino acids with an increase in the molar percent of alanine, glycine, proline, and histidine, but a decrease in arginine.

Trees showing cold injury symptoms in field studies were found to have much lower levels of prunasin in the bark. Cyanide released as the result of prunasin breakdown has the potential to cause severe tissue damage and may be an underlying cause of tissue death.

Introduction

Peach tree short life (PTSL) syndrome is a serious problem of peach production primarily in the southeastern United States. This area has over 9 million peach trees with an annual production of 350,000 MT, about half of the total production of fresh peaches in the United States. Tree death from PTSL varies each year. In recent years, tree losses have been about 2%, or 180,000 trees per year, representing $10 million in lifetime loss each year. Severe losses occurred in 1962 (300,000 trees in Georgia alone), 1973 (500,000 trees in Southeast), and 1984 (200,000 trees in Southeast).

Typical symptoms of PTSL are seen most often on trees 3-6 years old. Trees that looked healthy in the fall either fail to bloom or bloom and leaf out then collapse. All or part of the tree may die. Foliage of partially injured trees will appear chlorotic, and may recover if the weather is cool. Usually symptoms of cold injury (darkened cambium, loose bark, sour fermented smell) or bacterial canker caused by Pseudomonas syringae Van Hall (sunken discolored areas) will be seen on trunk and scaffold limbs (18). In our area, cold injury is more common than P. syringae damage. In more northern areas, Cystospora may invade damaged tissue. Damage is worst on the south (sunny) side of the tree and does not extend below the soil line. In many cases, suckers will come up under dead trees, indicating the roots are still alive. In many ways, the PTSL syndrome resembles classic cold damage seen in many crops. The trees do not remain as dormant as they should in late winter and early spring, and are subject to injury by normally non-lethal temperatures.

The presence of root suckers in some instances distinguishes death by PTSL from death by root rot caused by the similar fungi Clitocybe tabescens (Scop. ex Fr.) Bres. or Armillaria mellea (Vahl) Quel. (17). External symptoms are similar for PTSL and

Clitocybe root rot, and undoubtedly many trees said to have PTSL in fact had root rot, or perhaps both. Root rot is readily distinguished by tissue death occurring from the roots up. Cutting under the bark of the roots (and lower trunk in severe cases) reveals a characteristic white mycelial mat. Rarely one sees the mushroom fruiting bodies in the fall. Infected root pieces can remain viable in the soil for many years. Losses to Clitocybe root rot in South Carolina were estimated at 140,000 trees in 1983, a lifetime loss of over $10 million.

PTSL syndrome continues to limit peach production in the southeastern United States despite more than 30 years of research on the problem (22). "Predisposing" factors are now thought to include orchard site, Criconemella xenoplax (Raski) Luc & Raski (syn. Macroposthonia xenoplax or Criconomoides xenoplax), rootstock, cultural practices, and fluctuating winter temperatures (15,16,22,26). Little is known of the physiological and biochemical basis for this predisposition although changes in auxin levels have been suggested (1).

PTSL is most common in orchards where there was a previous planting of peaches although it sometimes occurs in virgin sites. In Georgia, which in 1928 had over 16 million peach trees, many sites have had multiple orchards over the last 100 years and virgin land is increasingly difficult to obtain. The harmful effect of a previous orchard apparently can last over 20 years, although it diminishes with time. The site effect is still unknown. It may relate to changes in soil fertility (toxicity or deficiency), structure (hardpans), or plant pathogenic organisms (nematodes, fungi, bacteria).

Recent work has shown that the C. xenoplax is a key factor in PTSL (14,15). This ectoparasite is common in the southeastern United States, especially where peaches have been planted repeatedly. Research in South Carolina showed trees grown in microplots (large closed-system underground pot) died of cold injury after 4 years of parasitism of C. xenoplax, while trees in uninfested soil survived (16). It is not known if other nematodes can similarly predispose peach trees to PTSL although Pratylenchus penetrans [(Cobb) Filip. and Stek.] has been reported to reduce cold-hardiness in cherry (3). Soil fumigation, especially post-plant, has been found to extend tree life dramatically apparently by reducing nematode populations (14,26).

Growth reduction of peach by C. xenoplax was demonstrated only after potted trees were kept in infested soil for 2.3 years (10). Subsequent research in California indicated 16 months exposure of potted trees to C. xenoplax could reduce fresh weight and increase susceptibility to bacterial canker (11). Criconemella xenoplax can produce a heat-labile auxin inactivating enzyme (23). When 1,2-dibromo-3-chloro-propane (DBCP) was available, regular pre- and post-plant soil fumigation kept C. xenoplax at low levels and prolonged peach tree life in the Southeast (22,26). This circumstantial evidence and recent work (16) indicate C. xenoplax plays a key role in tree death. Resistant rootstocks would be an attractive alternative to chemical fumigation, especially since nematicides currently available are less effective than DBCP.

Lovell and Nemaguard cultivars are the most common rootstocks for peach in the U.S. At one time, Lovell was the predominant drying peach in California so seed was

readily available for nursery use. Although trees on Lovell generally outlive those on Nemaguard in the Southeast particularly where C. xenoplax is prevalent, all are nonetheless subject to cold injury and tree death. Nemaguard, released by USDA in 1961, is resistant to root-knot nematode [Meloidogyne incognita (Kofoid & White) Chitwood and M. javanica (Treub) Chitwood] whereas Lovell is susceptible to root knot. Although Nemaguard was originally developed in Georgia, poor survival limits its use in the Southeast.

Although trees on Lovell may be longer-lived than those on Nemaguard, data to show Lovell rootstock is tolerant or resistant to C. xenoplax are inconclusive. Apparent differences in rate of population build-up on Lovell as compared to Nemaguard (26) may be more related to the faster growth rate of Nemaguard (25) than to inherent resistance or susceptibility. It is not clear whether the ideal rootstock would need to 1) resist or inhibit buildup of the C. xenoplax on its roots, 2) tolerate high levels of the nematode without growth reduction, 3) simply not predispose a scion to injury from cold or P. syringae (unrelated to 1 or 2), or 4) have some combination of these characteristics. Screening procedures for resistance and tolerance are relatively straightforward and currently underway. Screening for longevity requires an understanding of the physiology of predisposition so that it can be induced and measured under controlled conditions in a reasonable length of time. Although it is difficult to simulate fluctuating weather conditions and field cultural practices under greenhouse conditions, a comparison of Lovell and Nemaguard may provide clues to why Nemaguard is shorter-lived and how to screen for survival potential.

Various cultural practices can affect tree longevity. Generally, good horticultural practices enhance tree life. Proper site preparation, adequate liming to pH 6.5, sod-herbicide orchard floor maintenance (rather than disking), and adequate soil fertility all help reduce, but do not eliminate the PTSL problem. In some years, pruning in early winter will increase the severity of PTSL, so late winter pruning is recommended (14,20). In areas where PTSL occurs, trees on rootstocks of Lovell and Halford (old drying and canning peach cultivars from California) generally outlive trees on root-knot resistant rootstocks (2,22,26). Trees on root-knot resistant stocks, including Nemaguard, Shalil, Yunnan, and S-37, have consistently survived poorly. It is not known whether this poor survival is related to root-knot nematode resistance itself or to the similar origins of these lines. Where root-knot nematode is prevalent, Nemaguard may outperform Lovell, since Lovell is quite susceptible to root-knot.

The severity of PTSL in a given year apparently relates to weather. Years having severe losses are characterized by extreme fluctuations in temperature particularly in January and February (19). Late winter temperatures often exceed 20 C during the day, which warms the tree and soil. After several days of warm temperatures, a sudden drop overnight to sub-zero temperatures (-5 C for example) will often damage or kill the trees. Comparable low-temperatures earlier in the winter do not appear to be harmful.

Approach

USDA research at the Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA, directed at PTSL involves a cooperative team including breeder-horticulturist, pathologist, nematologist, and soil scientist. Several projects are in cooperation with scientists at adjacent universities, particularly in South Carolina and Georgia. Our research goal is to determine how the factors previously discussed alter tree physiology in such a way that trees are predisposed to cold injury. Ultimately, we would like to be able to screen efficiently for a rootstock that would increase tree life. Current emphasis is on measuring the response of trees to nematode feeding, since this key factor is more readily manipulated in controlled studies.

Although much is known about what predisposes trees to PTSL, little is known about how this occurs. Since a root-feeding nematode is implicated in altering the cold-hardiness of a scion, there must be chemical and physiological changes occurring throughout the plant. Only a few changes have been documented.

Current Research

Considerable evidence has accumulated demonstrating a significant involvement of C. xenoplax in PTSL. Yet, the chemical basis of this involvement is not understood nor changes in plant metabolism leading to increased cold sensitivity recognized. One measure of differences in plant metabolism is the level of free amino acids that appear as ninhydrin reactive compounds (NRC). Both NRC levels and specific amino acids have been related to cold hardiness (4,8,9) and nematode injury (5,6,7,13,21). Cyanide has been suggested to have a role in replant problems (12,24). Lovell and Nemaguard seedlings and herbaceous cuttings were grown for 8-13 months in soil with or without C. xenoplax and various parameters measured.

Experiment 1. The presence of C. xenoplax significantly reduced root fresh and dry weight on Lovell and Nemaguard seedlings (Table 1). NRC of roots decreased 47% and 41% while shoot NRC decreased 38% and 48% with nematodes for Lovell and Nemaguard, respectively. Rootstock effect differed only for stem diameter and root NRC (Table 1). No treatment x rootstock effects were significant.

Experiment 2. The presence of C. xenoplax on rooted cuttings of Lovell and Nemaguard significantly reduced root fresh and dry weight, and percent dry matter (Table 1). NRC of roots decreased 25% and 23% for Lovell and Nemaguard cuttings. Shoot NRC remained the same for Lovell but in Nemaguard decreased 31% in the presence of nematodes. Rootstocks differed in root dry weight and dry matter content.

Significant reductions in root fresh and dry weight, and percent dry matter occurred in the presence of C. xenoplax on Nemaguard seedlings (Table 1). Nematode effects on growth were greater in this test than in Experiment 1. The NRC of shoots decreased 36% and that of roots 14%.

The relative ratios of amino acids as well as the total concentration were altered by C. xenoplax in roots of both Lovell and Nemaguard cuttings (Table 2). The presence

of C. xenoplax increased the molar percent of alanine, glycine, and proline in particular, whereas arginine was decreased. Nemaguard seedlings generally showed the same pattern as cuttings, except for aspartic acid (Table 2). Total free amino acid concentration was reduced 53% in roots of Lovell with C. xenoplax whereas Nemaguard cuttings had total amino acids reduced 60% and Nemaguard seedlings had a 72% reduction in the presence of C. xenoplax.

The cyanogenic glucoside prunasin decreased significantly in stem tissue for both Lovell and Nemaguard herbaceous cuttings and Nemaguard seedlings infested with C. xenoplax (Table 3). Concentrations decreased less in Lovell stems (10%) than in stems of Nemaguard cuttings (49%) or seedlings (34%), respectively. Prunasin levels of root tissue were, however, significantly increased for all rootstocks in the presence of C. xenoplax.

Results of this study showed growth reduction resulting from long-term exposure to C. xenoplax. Nemaguard and Lovell showed similar growth reduction as a result of nematode infestation. Nematode population increase was also comparable for the two rootstocks. The poor performance of Nemaguard as a rootstock in the Southeast may not be due to its quality as a host for C. xenoplax, but its different physiological response to parasitism.

It is not unexpected that the presence of C. xenoplax reduced NRC and total free amino acid levels in the roots of infested plants. When total free amino acid levels are compared, it is obvious that the root chemistry is radically altered. It is not clear whether the nematode is removing NRCs, or whether NRC production is reduced or the compounds redirected. Except for Lovell cuttings, shoot NRC levels in both experiments were also reduced by the presence of C. xenoplax. These changes in shoot NRC levels indicate a response by the shoot to the presence of an ectoparasitic nematode on the roots.

Our results showing changes in relative molar ratios of specific amino acids in the roots of potted trees may provide clues to the predisposition caused by the nematode. Since C. xenoplax is implicated in PTSL, it is clear that the nematode-induced changes in root physiology must alter scion physiology to the point that normally non-lethal temperatures can cause tree injury. Additional work is needed to relate these results to field conditions.

The reported changes in prunasin levels in roots in the presence of C. xenoplax is of interest when considering changes in tree physiology. Prunasin is present in relatively high concentrations in bark and roots of peach trees, and has been found to be a potent inhibitor of nitrate reductase upon breakdown to mandelonitrile and cyanide.

These results demonstrate an ectoparasitic nematode C. xenoplax can cause biochemical changes in both the roots where they feed, as well as in the shoot. Additional work is needed to clarify the nature of the predisposition of peach trees to PTSL, determine the relationship of changes in amino acid and prunasin levels and predisposition, and develop an efficient rootstock screening technique for long-term survival.

A field study was conducted in the spring of 1984 during a severe PTSL outbreak. This study investigated the physiological responses of dying peach trees in PTSL orchards by comparison, chemically, to apparently healthy trees in the same orchard and to healthy trees in non-PTSL orchards. Factors known to contribute to PTSL such as pathogens, soils, environmental conditions, and cultural practices were monitored in each site and similarities and differences noted.

Tree losses in PTSL orchards ranged from 11 to 30 percent, whereas healthy orchards had only 0.3 to 1.5% tree loss due to PTSL. Overall, PTSL losses in Georgia in the spring of 1984 were 5%. Of the five orchards selected for this study and showing severe PTSL, four were on land previously planted to peaches. Recommended pruning practices (spring or summer pruning) were followed in three of the orchards. PTSL developed even in a summer pruned orchard with apparently no previous history of having peaches planted on the site (Table 4). Of the healthy orchards selected, one was summer pruned, two fall pruned and one spring pruned. C. xenoplax populations in these orchards were relatively low as compared to PTSL trees in the PTSL orchards and about equal to the apparently healthy trees from those orchards. The soil composition of all orchards was of the sandy loam type. With the exception of healthy Orchard 6, the pH of top soil and subsoil of healthy and PTSL orchards did not appear to differ greatly and were all within a range of 4.55 to 5.88.

Bark prunasin content on the south side of the PTSL trees was drastically reduced (0.1mg/cm^2 bark) as compared to the north side (0.5mg/cm^2 bark) of these trees. In apparently healthy trees in the same orchard, the prunasin content remained high (1mg/cm^2 bark) for both sides of the tree and was in the range found in trees from orchards considered healthy.

Reducing sugars were decreased by an average of 50% on the south side of the PTSL trees as compared to the south side of apparently healthy trees from the PTSL orchards, whereas the north side had an average decrease of 30%. Reducing sugars from trees in healthy orchards were somewhat lower than those found in the PTSL orchards and were the same on both sides of the tree.

Bacterial canker caused by P. syringae did not appear to be associated with PTSL in this study. Of the 60 samples from healthy and PTSL trees from three orchards, only eight were positive and these were randomly distributed throughout the samples.

All of the PTSL orchards in this study were well below the recommended soil pH of 6.5 (11,13). Healthy Orchard 6, pruned in the fall, had a pH of greater than 6.0 and a high C. xenoplax population; whereas Orchard 7, also pruned in the late fall, on Nemaguard rootstock, and planted on land previously in peaches, had a very low C. xenoplax population. These observations were consistent with previous reports indicating that low pH, land previously planted to peaches, and incorrect pruning practices, in combination with high C. xenoplax populations predispose trees to PTSL (15,16,22). Under field conditions it appears that a blend of factors including site, nematode populations, soil pH, and pruning practices interact to predispose the trees. In this study, summer pruning appears to be a contributing factor to PTSL predisposition when in combination with high C. xenoplax populations and low soil pH.

The primary injury to PTSL trees appeared to be from cold damage which occurred most severely on the south side of the trunk. Late-winter subzero temperatures, preceded by sustained warm periods, are thought to cause the cold damage to predisposed peach trees (19).

Measurements of chemical constituents of trunk samples of the south and north sides of PTSL trees and apparently healthy trees from PTSL orchards revealed obvious trends in the cold damaged tissue. The concentration of prunasin, a cyanogenic glucoside, was about 1-1.5 mg/cm^2 of bark on both the north and south side of the healthy trees, but only 0.5 mg/cm^2 on the north side and less than 0.1 mg/cm^2 on the south side of the PTSL trees. Reducing sugars decreased on both north and south sides of PTSL trees. The decrease in reducing sugar concentration of PTSL trees may be a result of fermentation by organisms which invade the dead and dying tissue.

It is thought that predisposing factors such as C. xenoplax, site factors (yet unknown), low soil pH, rootstocks, and incorrect pruning time reduce the cold hardiness of the tree trunk in late winter, mainly on the south side where solar radiation elevates temperatures higher than on the north side of the trunk. During subsequent periods of sub-zero weather, this susceptible area of trunk tissue suffers damage resulting in loss of cell membrane integrity. Our data does not allow us to determine if the loss of prunasin is the result of cold injury and tissue death, or if in fact, prunasin breakdown contributes to tissue death. If prunasin and certain enzymes come in contact, the degradation of prunasin and the release of cyanide could result.

The levels of prunasin found in healthy bark (1-1.5 mg/cm^2 of bark) could generate sufficient cyanide to severely damage plant tissue. However, it is not known whether prunasin breakdown occurs as a result of tissue injury by the cold temperatures, or whether prunasin breakdown generates cyanide which is at least partly responsible for the tissue damage itself. Answers to questions such as these may provide an understanding of the physiological basis of PTSL, and ultimately allow a solution to be developed for the problem.

Literature Cited

1. Carter, G. E., Jr. 1976. Effect of soil fumigation and pruning date on the indoleacetic acid content of peach trees in a short life site. HortScience 11:596-595.
2. Dozier, W. A., Jr., Knowles, J. W., Carlton, C. C., Rom, R. C., Arrington, E. H., Wehunt, E. J., Yadava, U. L., Doud, S. L., Ritchie, D. F., Clayton, C. N., Zehr, E. I., Gambrell, C. E., Britton, J. A., and Lockwood, D. W. 1984. Survival, growth, and yield of peach trees as affected by rootstocks. HortScience 19(1):26-30.
3. Edgerton, L. J., and Parker, K. G. 1958. Effect of nematode infestation and rootstock on cold hardiness of Montmorency cherry trees. Proc. Am. Soc. Hort. Sci. 72:134-138.
4. El-Mansy, H. I., and Walker, D. R. 1969. Seasonal fluctuations of amino acids, organic acids, and simple sugars in 'Elberta' peach and 'Chinese' apricot flower

buds during and after rest. J. Am. Soc. Hortic. Sci. 94:184-192.
5. Epstein, E., and Cohn, E. 1971. Biochemical changes in terminal root galls caused by an ectoparasitic nematode, Longidorus africanus: Amino acids. J. Nematol. 3:334-340.
6. Feldman, A. W., and Hanks, R. W. 1964. Quantitative changes in the free and protein amino acids in roots of healthy, Radopholus similis-infected, and "recovered" grapefruit seedlings. Phyto- pathology 54:1210- 1215.
7. Gommers, F. J., and Dropkin, V. H. 1977. Quantitative histo- chemistry of nematode-induced transfer cells. Phytopathology 67: 869-873.
8. Holubowicz, T., and Boe, A. A. 1970. Correlation between hardiness and free amino acid content of apple seedlings treated with gibberellic acid and abscisic acid. J. Am. Soc. Hortic. Sci. 95:85- 88.
9. Lasheen, A. M., and Chaplin, C. E. 1971. Biochemical comparison of seasonal variation in three peach cultivars differing in cold hardiness. J. Amer. Soc. Hortic. Sci. 96:154-159.
10. Lownsbery, B. F., English, H., Moody, E. H., and Schick, F. J. 1973. Criconemoides xenoplax experimentally associated with a disease of peach trees. Phytopathology 63:994-997.
11. Lownsbery, B. F., English, H., Noel, G. R., and Schick, F. J. 1977. Influence of Nemaguard and Lovell rootstocks and Macroposthonia xenoplax on bacterial canker of peach. J. Nematol. 9:221-224.
12. Mizutani, F. 1980. Studies on the replant problem and water tolerance of peach trees. Mem. Coll. Agr. Ehime Univ. 24:115-198 (in Japanese).
13. Mohanty, K. C., and Das, S. N. 1976. Free amino acids in the roots of finger-millet plants infected with ring nematodes. Indian Phytopathology 29:434-436.
14. Nesmith, W. C., and Dowler, W. M. 1975. Soil fumigation and fall pruning related to peach tree short life. Phytopathology 65:277- 280.
15. Nesmith, W. C., Zehr, E. I., and Dowler, W. M. 1981. Association of Macroposthonia xenoplax and Scutellonema brachyurum with the peach short life syndrome. J. Nematol. 13:220-225.
16. Nyczepir, A. P., Zehr, E. I., Lewis, S. A., and Harshman, D. C. 1983. Short life of peach trees induced by Criconemella xenoplax. Plant Dis. 67:507-508.
17. Petersen, D. H. 1961. The pathogenic relationship of Clitocybe tabescens to peach trees. Phytopathology 51:819-823.
18. Petersen, D. H., and Dowler, W. M. 1965. Bacterial canker of stone fruits in the southeastern United States. Plant Dis. Reptr. 49(8):701- 702.
19. Prince, V. E. 1966. Winter injury to peach trees in central Georgia. Proc. Am. Soc. Hort. Sci. 88:190-196.
20. Prince, V. E., and Horton, B. D. 1972. Influence of pruning at various dates on peach tree mortality. J. Am. Soc. Hort. Sci. 97(3):303-305.
21. Poehling, H. M., Wyss, U., and Neuhoff, V. 1980. Microanalysis of free amino acids in the aseptic host-parasite system: Ficus carica-Xiphinema index (nematode). Physio. Plant Path. 16:49-61.
22. Ritchie, D. F., and Clayton, C. N. 1981. Peach tree short life: a complex of interacting factors. Plant Dis. 65:462-469.
23. Viglierchio, D. R., and Mjuge, S. G. 1976. Auxin inactivation systems of nemic origin. Nematologica 21:471-475.
24. Ward, G. M., and Durkee, A. B. 1956. The peach replant problem in Ontario.

III. Amygdalin contents of peach tree tissue. Can. J. Bor. 34:419-422.
25. Werner, D. J. and Young, E. 1982. Short-term growth analysis of 'Lovell' and 'Nemaguard' peach rootstocks. J. Hort. Sci. 57:377- 381.
26. Zehr, E. I., Miller, R. W., and Smith, F. H. 1976. Soil fumigation and peach rootstocks for protection against peach tree short-life. Phytopathology 66:689-694.

Table 1. Effects of Criconemella xenoplax (Cx) on growth and ninhydrin reactive compounds (NRC) of seedlings and rooted cuttings of Lovell and Nemaguard peach rootstocks.

Rootstock	Cx[a]	Stem diam (mm)	Root fresh weight (g)	Root dry weight (g)	Root % dry matter	NRC[b] shoot	NRC[b] root	Number Cx/150 cm^3 soil
Experiment 1, Seedlings								
Lovell	-	8.6	132	46	35	0.53	1.30	0
Lovell	+	7.8	115	34	30	0.33	0.69	1100
Nemaguard	-	8.8	129	43	33	0.56	1.82	0
Nemaguard	+	8.9	117	40	34	0.29	1.08	1400
Significant Effects								
Cx			+	*		**	**	
Rootstock		*					*	
Experiment 2, Herbaceous Cuttings								
Lovell	-	7.7	24	7.5	31	.64	1.73	0
Lovell	+	6.6	14	3.3	25	.64	1.30	6800
Nemaguard	-	7.5	24	6.7	27	.58	1.57	0
Nemaguard	+	7.1	17	4.0	23	.40	1.22	7900
Experiment 2, Seedlings								
Nemaguard	-	7.8	39	14.7	38	.69	1.20	0
Nemaguard	+	7.9	27	7.7	29	.44	1.03	18,300
Significant Effects[c]								
Cx			**	**	**			
Rootstock			*	**	**	**	+	**

a/ With (+) or without (-) Cx for 13 months (Experiment 1) or 8 months (Experiment 2).

b/ Ninhydrin reactive compounds as mM glycine equivalent/g dry weight.

c/ Indicates significance of F-test from analysis of variance at the 10% (+), 5% (*), or 1% (**) levels. Means over 8 blocks (Expt. 1) or 6 blocks (Expt. 2).

Table 2. Effect of Criconemella xenoplax (Cx) on amino acid composition in roots of Lovell and Nemaguard peach rootstocks after 8 months.

Amino Acid	Rootstock/Treatment[a]					
	Lovell cutting		Nemaguard cutting		Nemaguard seedling	
	-Cx	+Cx	-Cx	+Cx	-Cx	+Cx
Ala	6.7 ± 0.9[b]	12.7 ± 0.6	6.6 ± 1.7	10.3 ± 1.1	6.8 ± 1.0	14.3 ± 3.2
Gly	0.9 ± 0.1	1.8 ± 0.3	1.0 ± 0.6	1.9 ± 0.6	0.5 ± 0.1	2.5 ± 0.1
Pro	1.5 ± 0.2	7.4 ± 2.2	2.0 ± 0.6	8.3 ± 1.1	1.7 ± 0.1	9.5 ± 5.5
Thr	1.2 ± 0.6	2.2 ± 0.5	1.7 ± 0.3	3.1 ± 1.6	2.2 ± 0.6	2.9
Ser	8.1 ± 0.3	12.3 ± 3.6	7.9 ± 3.2	10.4 ± 2.5	2.6 ± 1.1	6.7 ± 6.5
Asp	24.6 ± 3.0	17.4 ± 0.2	22.9 ± 5.8	15.1 ± 7.5	21.3 ± 4.2	27.9 ± 11.4
Phe	3.6 ± 1.0	7.8 ± 1.6	6.0 ± 1.7	6.5 ± 2.3	4.1 ± 1.5	6.9 ± 2.6
Glu	0.7 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	1.0 ± 0.4	0.7 ± 0.9	0.6 ± 0.2
Tyr	0.6 ± 0.1	2.0 ± 1.4	0.3 ± 0.1	0.5 ± 0.2	1.0 ± 0.3	2.5 ± 1.3
Arg	52.4 ± 2.3	35.5 ± 1.6	50.8 ± 13.8	33.0 ± 5.6	52.6 ± 4.5	11.2 ± 6.0
Total[c]	37.0 ± 4.3	17.3 ± 1.7	38.7 ± 12.1	15.5 ± 2.3	44.9 ± 18.4	12.4 ± 4.1

a/ Denotes presence (+Cx) or absence (-Cx) of C. xenoplax on seedlings or rooted cuttings.

b/ Mean molar percent ± s.e. of triplicate determinations for each treatment.

c/ Total identified as moles/g dry weight of roots.

Table 3. The effect of 8 months exposure to Criconemella xenoplax (Cx) on the cyanogenic glucoside prunasin in peach seedlings and rooted herbaceous cuttings.

Rootstock	Cx	Mg prunasin/ g stem dry weight + s.e.	Mg prunasin/ g root dry weight + s.e.
Herbaceous cuttings			
Lovell	-	6.7 ± 1.0	21.5 ± 2.7
Lovell	+	6.0 ± 1.0	26.2 ± 1.9
Nemaguard	-	5.2 ± 0.5	16.8 ± 4.0
Nemaguard	+	3.1 ± 0.3	23.8 ± 2.5
Seedlings			
Nemaguard	-	6.4 ± 1.0	15.9 ± 1.5
Nemaguard	+	4.2 ± 0.6	20.4 ± 1.4
Significant effects[b]			
Cx		*	**
Rootstock		*	+

a/ Mean of 6 determinations per treatment.

b/ Significance of F-test at the 10% (+), 5% (*), or 1% (**) levels.

Table 4. Orchard history of selected PTSL and healthy orchards.

Orchard type	Age (yrs)	Scion/ Rootstock	Previous peach land/last orchard[a]	Cultural practices
1 PTSL	9	Harvester/ Lovell	yes/5 yrs	pruned: Aug 1983 touchup: Mar 1984 irrigated girdled
2 PTSL	6	Loring/Lovell	yes/2 yrs	pruned: Nov 1983
3 PTSL	3	Brighton/Lovell	yes/1 yr	pruned: Nov 1983
4 PTSL	7	Redskin/Lovell	yes/16 yrs	pruned: Mar 1984
5 PTSL	12	Coronet/Lovell	no	pruned: Aug 1983 touchup: Jun 1984
6 Healthy	9	Harvester/Lovell	no	pruned: Nov 1983
7 Healthy	6	Loring/Nemaguard	yes/15 yrs	pruned: Dec 1983
8 Healthy	7	Redglobe/Lovell	no	pruned: Mar 1984
9 Healthy	9	Harvester/Lovell	no	pruned: Aug 1983

a/ Years between previous orchard and this planting.

PEACH TREE FUNGAL GUMMOSIS

P. L. Pusey, C. C. Reilly, and W. R. Okie
USDA/ARS, S.E. Fruit & Tree Nut Research Laboratory
Byron, GA 31008

Abstract

Peach tree fungal gummosis was first observed near Fort Valley, Georgia in the early 1960's. Later, Weaver (1974) described disease symptoms and determined that the causal agent was Botryosphaeria dothidea. The fungi, B. obtusa and B. rhodina have also been implicated in the disease. Our investigations reported here verified that B. dothidea is the cause of "swollen" and sunken areas around lenticels and gum exudation from them as reported earlier by Weaver (1974). Our studies also indicate that a certain strain(s) of B. dothidea is involved in peach fungal gummosis. In non-wound inoculations of potted peach trees a B. dothidea isolate from peach became established in lenticels and eventually invaded phelloderm and phloem tissues, whereas an isolate from plum appeared to only persist in the phellem of lenticels and did not invade living tissue. All twenty-eight isolates of B. dothidea used in wound inoculations of peach induced gumming during a three month period following inoculation. After 15 months, amount of gum exudation was moderate to high for all 13 peach isolates and 5 non-peach isolates from within the reported peach fungal gummosis area. At this time, however, the remaining 10 isolates, which included all isolates from outside the gummosis area (1 peach and 5 non-peach), had ceased or nearly ceased to induce gumming. During the 1984 season, airborne ascospores of Botryosphaeria spp. from peach prunings were detected in greatest numbers in April. Although counts of B. obtusa conidia in January and February were relatively high, B. dothidea conidia counts were very low and did not increase until March. In a spring fungicide test, Difolatan was shown to effectively reduce natural infection of pruning wounds by B. dothidea and B. obtusa.

Introduction

Severe gumming of peach [Prunus persica (L.) Batsch] trees was apparently first noticed in an orchard near Fort Valley, Georgia, sometime in the early 1960's according to a personal communication with V. E. Prince (5). This condition was later found to be the result of a fungal disease which has since spread to a number of the major peach-producing areas of the southeastern United States (Fig. 1). By 1981, peach tree fungal gummosis was present in Georgia, Alabama, Florida and Louisiana (5), and more recently it has been found in Mississippi (R. A. Haygood, personal communication). It has also appeared in a few areas of South Carolina, but the diseased trees were destroyed by burning (R. W. Miller, personal communication).

The first published report of peach gummosis in the Southeast was by Weaver (8) in 1974. Symptoms described by Weaver included sunken necrotic lesions (1-2 cm in diameter) in bark around lenticels of trunks and scaffold limbs and gum exudation from these lenticels. Infected lenticels of young branches became "swollen", but generally did not exude gum. The fungus, Botryosphaeria dothidea (Moug. ex Fr.) Ces. & de Not. (B. ribis Gross & Dugg.), was consistently isolated from the diseased bark. In initial attempts to reproduce the gummosis symptoms, wound inoculations with B. dothidea resulted in the development of a gummy canker after 3 months, and after 18 months, infected lenticel and gum deposits typical of gummosis were observed up to a distance of 40 cm below the point of inoculation. In June, 1977, Weaver (9) exposed

1-3 year old tree branches to B. dothidea spores produced in pure culture. No wounds were made. Three months later, swollen lenticels and sunken necrotic lesions in bark beneath lenticels were observed. Gumming, however, was not observed during the 5-month-long study.

Progress in research on peach gummosis appeared to have slowed when Weaver left Georgia. As other workers have studied the disease, questions have arisen as to what fungus or fungi are involved. Reilly and Okie (5), in their 1981 survey, reported that B. dothidea was frequently isolated from bark below gum exudates, although B. obtusa (Schw.) Shoemaker was occasionally isolated. In contrast to this, Britton and Hendrix (1) isolated B. obtusa more frequently than B. dothidea from cankers. A third fungus, B. rhodina (Berk. and Curt.) Arx, was also isolated from cankers, but this was relatively rare. The 1981 survey was conducted in the fall. Britton and Hendrix made isolations from cankers throughout the year and found population shifts in the 3 fungi.

The role of lenticels as infection ports and as an important part of the gummosis syndrome has received less emphasis than one would have expected. This presents a pertinent question: Are lenticels unimportant or have they simply been overlooked by those currently working on gummosis?

There are other fundamental questions concerning gummosis which we are attempting to answer. We know that both B. dothidea and B. obtusa have a wide distribution in the United States. As early as the 1920's, B. dothidea was isolated from peach in Florida and from apple near Fort Valley, GA (6), yet the severe gummosis of trees was not noticed until the early 1960's. Have virulent strains of one or both of these fungi recently developed or has a change in environmental factors or cultural practices in the Southeast resulted in an increased susceptibility of peach trees to the disease? We know very little of the epidemiology of gummosis. The source of inoculum for early disease development in an orchard remains unknown as well as the means of dissemination within the tree and from tree to tree. It appears that fungal gummosis reduces fruit yield, tree vigor, and longevity. However, we have been unable to determine the economic importance of the disease because orchards are generally uniformly infected, and diseased and non-diseased trees cannot be compared. The most urgent concern arising from the disease is to find an economical control which would prevent the spread of gummosis into new orchards.

Current Research

Several studies of gummosis are being conducted currently at Byron. Inoculations of trees in the orchard and greenhouse have been done mainly by introducing fungal isolates into wounds. In the past year we have been exposing trees to the fungal spores without wounding. This was done to verify whether B. dothidea or other Botryosphaeria spp. could enter the tree through lenticels and establish infections as shown by Weaver (8,9).

Non-wound Inoculations

In June, 1983, an experiment was set up to determine if any of the Botryosphaeria spp. isolated from peach can initiate infections at lenticels. One peach isolate of each of the fungi, B. obtusa and B. rhodina, and two isolates of B. dothidea (one from peach and one from plum) were used to inoculate 2-year-old 'Sunland' peach trees in a greenhouse. Each isolate as a suspension of 10^5 spores per ml was sprayed onto 25 cm of the main stem (1.0-1.5 cm dia) of 4 trees. The inoculated area was wrapped with moist cheese cloth and parafilm. Bark above and below the treated area was marked with latex paint before removing wrappings 6 days later.

Inoculated trees developed no apparent symptoms during the initial 4 months. They were transferred to a lath house and maintained through the winter and spring. Thirteen months after trees were inoculated with the peach isolate of B. dothidea, they showed profuse gumming at a number of points along the inoculated portion of the stem (Fig.2). Close examination revealed many bumps or raised areas (2-4 mm dia.) and a few sunken areas (6-8mm dia.), all with lenticels in the center. When the outer bark was removed, raised areas frequently had necrotic spots 1-2 mm in diameter surrounded by apparently healthy tissue which protruded above normal bark. The entire area immediately interior to the phellem of sunken lesions was necrotic. Generally, brown discoloration extended less than 1 mm into the phloem. Occasionally, however, some brown streaking extended into the xylem below sunken lesions.

Trees inoculated with peach isolates of B. obtusa and B. rhodina and the plum isolate of B. dothidea had no visible disease symptoms. These inoculated trees were in no way noticeably different from non-inoculated control trees.

It was even more interesting to find that all of the fungi, including those which did not produce symptoms, were recovered from 50% of the lenticels. Ten isolations were attempted for each tree. Bark was surface sterilized with 2% sodium hypochlorite for 2 minutes. The outer bark of trees inoculated with the peach isolate of B. dothidea was removed and tissue from the necrotic/healthy interface was transferred to a solid medium. From all the other trees, which appeared healthy, tissue was taken from below outer bark tissue at the interface of the phellem of lenticels and surrounding live tissue. Recovery was 56% for the B. dothidea from plum, 58% for B. obtusa, and 60% for B. rhodina. No Botryosphaeria spp. were isolated from any of the 4 non-inoculated control trees.

Host Response to Fungal Strains

The possibility that a certain strain(s) of B. dothidea is responsible for fungal gummosis was investigated by inoculating trees with various isolates of the fungus. In May, 1983, 28 isolates of B. dothidea from peach and other tree hosts were used to inoculate wounds of 2-year-old 'Sunland' peach trees in the greenhouse. One isolate of B. rhodina from peach was also included. Of the B. dothidea isolates, 13 were obtained from peach trees within the reported fungal gummosis area, 1 was from peach

in West Virginia, 9 were from woody hosts other than peach but located in the peach fungal gummosis area, and 5 were from other hosts outside the gummosis area. The non-peach isolates were from apricot, species of plum and cherry, blueberry, apple, holly, and sassafras. Trees were wounded with pliers at 2 levels on the woody stem and 3 levels on a younger, more succulent branch. Both sides of the stem or branch wounded by the pliers were brushed with a fungal spore suspension (10^5 spores per ml). Six trees per fungal isolate were inoculated and rated periodically for gum exudation.

Two weeks after inoculation, B. dothidea isolates that induced the most gumming were from hosts other than peach, and included isolates from apple, blueberry, and cherry. The gum rating for the B. rhodina isolate was also relatively high. At 3 months relative differences among isolates were about the same as in the 2 week rating, but average gum ratings were higher overall. All 28 B. dothidea isolates induced gumming during the 1983 season. After 15 months, amount of gum exudation was moderate to high for all 13 peach isolates and 5 non-peach isolates from within the reported peach fungal gummosis area. At this time, however, the remaining 10 isolates, which included all isolates from outside the gummosis area (1 peach and 5 non-peach), had ceased or nearly ceased to induce gumming. Gumming induced by B. rhodina had also nearly stopped.

Because of the possibility of lenticel infections, trees were examined for diseased lenticels 17 months after wound inoculation. It was found that 93% of trees inoculated with the 13 peach isolates obtained from trees within the gummosis area had diseased lenticels, whereas none of the trees inoculated with the one peach isolate from outside the gummosis area showed symptoms involving lenticels. Diseased lenticels were observed on 39% of trees inoculated with non-peach isolates from the gummosis area and 12% of trees inoculated with non-peach isolates from outside the gummosis area. Inoculations with peach isolates from the gummosis area resulted in the greatest amount of tree death (46%) at 17 months in this study.

In September of 1984, essentially the same isolates of B. dothidea were used to inoculate both potted trees and 2-yr-old orchard trees by brushing spores onto non-wounded bark. Results are not expected until the 1985 season.

Monitoring of Fungal Spores

To gain an understanding of the epidemiology of fungal gummosis, the dissemination of sexual and asexual spores of Botryosphaeria spp. is being studied. Starting in June, 1982, airborne ascospores were trapped with a Burkard 7-day recording volumetric spore trap (Burkard Scientific Ltd., Rickmansworth, Hertfordshire, England). The trap was placed near a weather station at Byron and surrounded by 4 cages built in the manner described by Sutton (7) which contained naturally infected peach prunings. The orifice of the trap was about 45 cm from the ground and about 71 cm from each cage. A mixture of twigs pruned from trees in different orchards during the past 2 years were placed in the cages initially; however, in January, 1984, these were replaced with peach prunings that had been collected from a single orchard and piled

near its edge in March, 1983.

The Burkard trap was adjusted to sample 10 liters of air per minute. The Melinex tape from the trap was treated with acid fuchsin in lactophenol before making spore counts.

Few airborne ascospores of Botryosphaeria spp. were detected with the Burkard trap between June and December of 1983. The greatest mean hourly concentration for B. dothidea for a single day was 2.08 ascospores/m^3 air/hour, recorded in early June. We have been unable to distinguish ascospores of B. obtusa and B. rhodina. The highest concentrations of ascospores of this type were between 6 and 9 ascospores/m^3 air/hour of 4 different days in August and September. In 1984, a dramatic increase in the concentration of both types of ascospores was detected in April. Mean hourly concentration of B. dothidea ascospores for individual days during a 2 week period in April ranged from 7 to 165 ascospores/m^3 air/hour. In this same period counts of B. obtusa or B. rhodina ascospores ranged from 0 to 15 ascospores/M^3 air/hour. After April, ascospore concentrations were relatively low.

Water dispersal of Botryosphaeria ascospores and conidia has been studied since March, 1983. Rainwater was collected in funnel traps placed beneath; 1) cages of prunings near the weather station, and 2) 6-year-old diseased peach trees ('Redglobe)'. A 10 ml solution of 5% copper sulfate was added to each collecting bottle to prevent spore germination. Bottles were changed weekly.

Rain traps used in the 2 situations described above were similar to one another in results produced. We do not have data for the months of October, November, and December, either because of dry weather or failure to collect the rain water. Conidia of all 3 Botryosphaeria spp. were detected during all other months of the year, except B. Rhodina conidia were not detected in February or September. Although numbers of B. obtusa conidia in January and February were high, numbers of B. dothidea conidia were very low during these months and did not increase until March. Both types of ascospores were detected in water from March through September.

Fungicide Test

Although our knowledge of fungal gummosis is limited at this time, control measures, including the use of fungicides, are being considered. During the 1983 season, fresh pruning wounds of 6-year-old 'Blake' peach trees were treated with the following fungicides and rates and were later inoculated with B. dothidea. Fungicides tested were chlorothalonil (Bravo 6F, 7.5 ml/L), captafol (Difolatan 4F, 5ml/L), liquid lime sulfur (120 ml/L), and a captan/benomyl mixture (Orthocide 50 WP, 3.6 g/L and Benlate 50 WP, 0.9 g/L). The chemicals were sprayed onto wounds immediately following pruning. A 10^5 spore per ml suspension of B. dothidea was used to inoculate wounds the day that fungicides were applied or 1 week or 2 weeks following the fungicide treatment. One set of trees for each fungicide was left uninoculated. Trees were pruned and treated in March and August. For each treatment at each pruning time, there were 4 replications of 2 trees with 8 pruning cuts per tree. Wounds were rated

for gum exudation at 8 weeks and reisolations of fungi were made 4 months following pruning.

In the March test, average gum ratings (0-4) for wounds treated with fungicides and inoculated the same day or inoculated one week later were all lower than the untreated checks. Both untreated and treated wounds inoculated 2 weeks after pruning produced relatively low amounts of gum and fungicides were not shown to reduce gumming. Differences between the treated and untreated, inoculated wounds were greater in March than in August, but in either case results were not impressive. Wounds may have been overwhelmed by the fungus. Observations of uninoculated wounds in the March test did, however, reveal that Difolatan was effective in reducing natural infection of B. dothidea and B. obtusa. Botryosphaeria dothidea was isolated from 29% of untreated wounds and 0% of wounds treated with Difolatan. Similarly, B. obtusa was isolated from 23% of untreated wounds and 3% of Difolatan treated wounds.

General Discussion

Results of the non-wound inoculation test confirmed earlier reports by Weaver (8,9) and prompted us recently to visit nearby orchards to determine the frequency of disease symptoms involving lenticels. Several field trips were required before we became proficient in routinely detecting these symptoms. Diseased lenticels were far more apparent on 3 and 4 year old trees than on trees of other ages. Trees younger than 3-4 years had either not become infected or had not yet expressed symptoms. Bark of older tree trunks and scaffold branches was thicker and it was difficult to determine whether infection that led to gum exudation had occurred at lenticels. Necrotic areas beneath gum deposits of older bark were frequently irregular in shape and sometimes extended to the xylem. However, abnormal lenticels were easily found on young branches of older trees. Raised areas associated with lenticels were more common on 1 to 2-year-old bark and sunken lesions were more common on bark 3 to 4-year-old bark. Bark samples with lenticel infections were recently collected from 7 different orchards. Botryosphaeria dothidea was isolated from 51 out of 52 samples. Pycnidia of B. dothidea were commonly found in diseased lenticels and perithecia of this fungus were found occasionally. Based upon the non-wound inoculations of potted trees and these observations, we believe that lenticel infections caused by B. dothidea are an important part of peach tree fungal gummosis.

The non-wound inoculation experiment and the study involving inoculations with 28 B. dothidea isolates indicate that a unique strain(s) of B. dothidea is associated with peach fungal gummosis. In the former test, the B. dothidea isolate obtained from peach within the gummosis area was able to establish itself in lenticels and eventually invade phelloderm and phloem tissues, whereas the plum isolate of B. dothidea appeared to persist only in the phellem of lenticels and did not invade living tissue. The fact that B. dothidea isolates from within the peach fungal gummosis area continued to induce gumming after one year while others did not, suggests that these isolates are more virulent and capable of continued advancement in healthy bark tissue.

Recently when attention was drawn to the role of lenticels in fungal gummosis, anatomical studies were initiated. Bark tissues collected periodically from trees inoculated in September are being fixed and prepared for sectioning. We are also interested in developing studies on the physiology of B. dothidea and the host-parasite interaction.

Airborne ascospores detected in high numbers in April, 1984, might be involved in the spread of fungal gummosis to young plantings of peach trees. Generally, orchards are uniformly infected. Gummosis symptoms are no more severe at the edge of a young orchard which is nearest to an older diseased orchard than in the center. It is easier to envision this happening with airborne spores as the initial inoculum during early disease development than with water dispersed spores. Airborne ascospores could occasionally infect pruning wounds directly, but more importantly the spores might land on pruning wood on the orchard floor, resulting in rapid colonization by the fungus and a massive build-up of spore inoculum. At this point, spread by water or insects may be the most important dispersal mechanism.

Control measures must include good sanitation practices. We know from spore trapping studies that the Botryosphaeria fungi can rapidly colonize twigs and branches pruned from trees and develop mature fruiting bodies after only 2-4 weeks. As long as bark remains intact on the dead prunings, spores can be released. Prunings must be removed from the orchard or chopped with a flail mower to separate bark from wood and bring about faster decomposition.

Field observations suggest that pruning wounds are frequently primary sites of infection. Abnormal lenticels are commonly found below these infections and we presume that they are the result of secondary infection. To reduce the possibility of pruning wounds becoming infected, it might be best to prune trees in late winter when levels of B. dothidea ascospores and conidia are low. However, B. obtusa conidia, which are at relatively high levels during this period, might infect wounds and contribute to easier B. dothidea entry and establishment later on.

The Botryosphaeria fungi are notorious for being stress pathogens. Conner (2) reported that B. dothidea can infect apple trees through lenticels, but the fungus does not move into the cortex until moisture stress develops. In a cooperative project with workers at the University of Georgia and Clemson University, cultural practices will be studied in relation to stress and predisposition of trees to fungal gummosis.

Whether fungicides can be used to control this disease is yet to be determined. We are hopeful that Difolatan will be effective, however it has not been approved for use on peach trees.

Studies on possible resistance in peach to fungal gummosis are also being conducted. Okie and Reilly (4) reported that 'Eagle Beak', a plant introduction from China, showed very little natural infection and exhibited less gumming than other lines when artificially inoculated. Daniell and Chandler (3) reported high field resistance for 'Harbrite'. In 1983, both of these cultivars produced less gum than most other

inoculated lines in response to B. dothidea and B. obtusa.

Literature Cited

1. Britton, K. O., and Hendrix, F. F. 1982. Three species of Botryo- sphaeria cause peach tree gummosis in Georgia. Plant Disease 66:1120-1121.
2. Conner, S. R. 1968. Canker formation on apple bark by Botryo- sphaeria ribis. Ph.D. thesis, University of Delaware, Newark. 157 pp.
3. Daniell, J. W., and Chandler, W. A. 1982. Field resistance of peach cultivars to gummosis disease. HortScience 17:375-376.
4. Okie, W. R., and Reilly, C. C. 1983. Reaction of peach and nectarine cultivars and selections to infection by Botryosphaeria dothidea. J. Amer. Soc. Hort. Sci. 108:176-179.
5. Reilly, C. C., and Okie, W. R. 1982. Distribution in the southeastern United States of peach tree fungal gummosis caused by Botryosphaeria dothidea. Plant Disease 66:158-161.
6. Stevens, N. E. 1926. Occurrence of the current cane blight fungus on numerous hosts in the southern states. Mycologia 18:278-282.
7. Sutton, T. B. 1981. Production and dispersal of ascospores and conidia of Physalospora obtusa and Botryosphaeria dothidea in apple orchards. Phytopathology 71:584-589.
8. Weaver, D. J. 1974. A gummosis disease of peach trees caused by Botryosphaeria dothidea. Phytopathology 64:1429-1432.
9. Weaver, D. J. 1979. Role of conidia of Botryosphaeria dothidea in the natural spread of peach tree gummosis. Phytopathology 69:330-334.

Figure Legends

Fig. 1. Distribution of fungal peach tree gummosis based on 1981 survey (5) and personal communications. In the few areas of South Carolina where the disease was detected, trees were rogued (R. W. Miller, personal communication).

Fig. 2. Response of potted peach trees to non-wound stem inoculations with spores of Botryosphaeria dothidea isolates. One tree (left) was inoculated with an isolate obtained from orchard trees exhibiting symptoms of fungal peach tree gummosis and the other (right) was inoculated with an isolate obtained from plum.

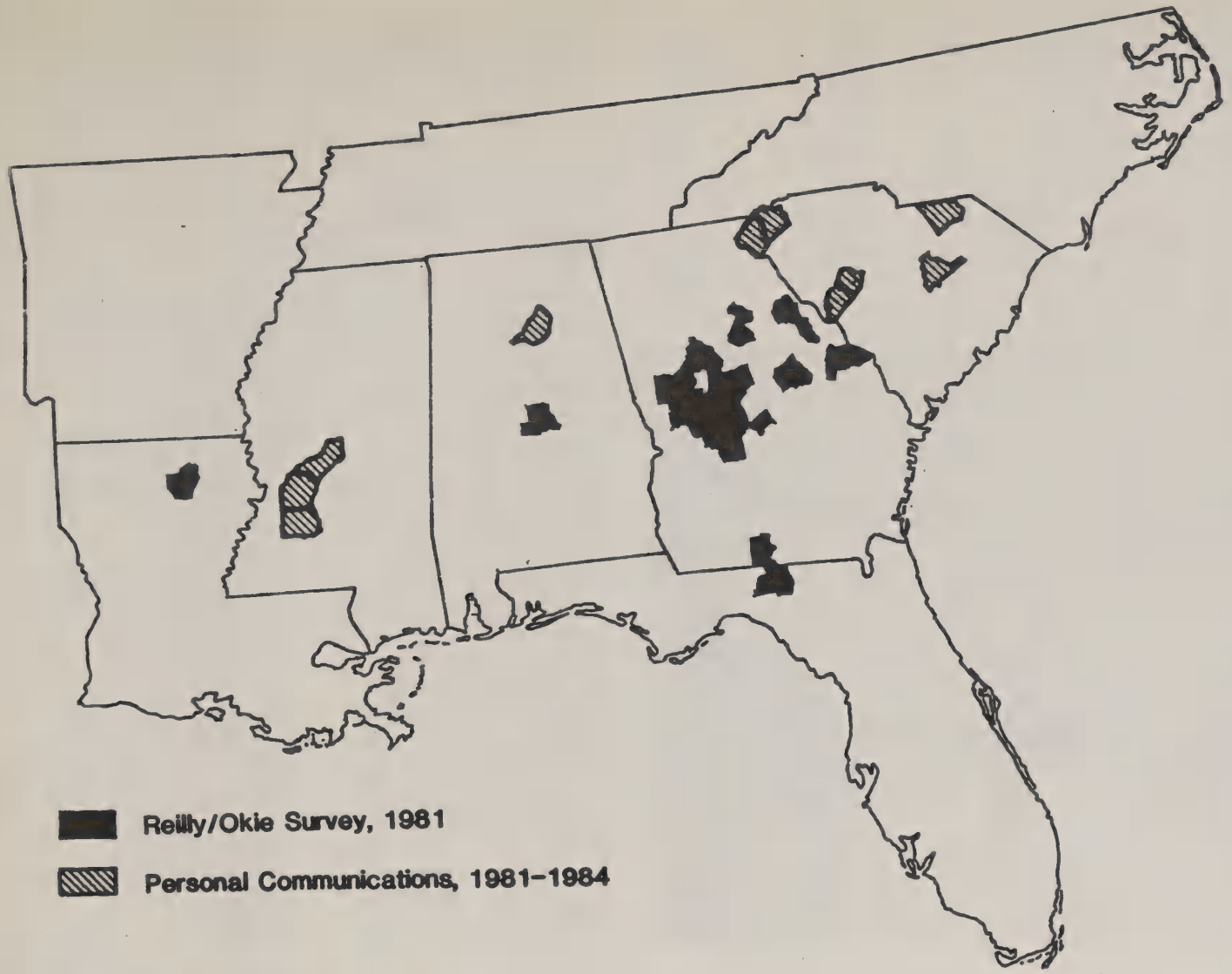


Figure 1. Distribution of fungal peach tree gummosis on 1981 survey (5) and personal communications. In the few areas of South Carolina where the disease was detected, trees were roughed (R. W. Miller, personal communication).

Figure 2. Response of potted peach trees to nonwound stem inoculations with spores of Botryosphaeria dothidea isolates. One tree (left) was inoculated with an isolate obtained from orchard trees exhibiting symptoms of fungal peach tree gummosis and the other (right) was inoculated with an isolate obtained from plum.

STUDIES OF THE BIOLOGY AND CONTROL OF PEACH GUMMOSIS

Kerry O. Britton and Floyd F. Hendrix
Department of Plant Pathology
University of Georgia, Athens 30602

Abstract

Most gummosis cankers contain three species of Botryosphaeria. Seasonal shifts in the species active in the cankers recurred over two years studied. Botryosphaeria dothidea predominates in summer, and B. obtusa in fall and winter. A third species, B. rhodina is active in early summer and fall, but is isolated in low numbers. Of these, only B. obtusa infects through buds as they open. All three species can infect through wounds, and produce indistinguishable symptoms. Histological studies reveal intercellular invasion of cortical tissues and ray parenchyma, especially in the zone of ray dilation. Xylem beneath cankers typically develops cavities, and vessel elements accumulate gum and tyloses.

In surveys conducted in 1981-82 we isolated considerably more Botryosphaeria obtusa from gummosis cankers than B. dothidea. Inoculations of wounds with these three species produce indistinguishable symptoms. Botryosphaeria obtusa is only a weak canker organism in Georgia (7), but it does sporulate in Georgia in Dec-Feb, which is early enough to infect apples through buds at silver tip (1). We observed that approximately 7% of the buds in a severely infected peach orchard failed to open, and we began to examine the possibility that these buds served as an infection court for this organism.

Isolations from symptomless buds for four years have shown the same trend of increasing infestation Dec through Feb, and much smaller increases in isolation rates from surface-sterilized buds.

Inoculations of potted trees increased spring bud mortality when B. obtusa or B. rhodina conidia were applied in Oct, (20 and 33%, respectively) compared with control and B. dothidea (6 and 12%, respectively). B. rhodina doesn't sporulate early enough to infest buds in the field.

For three years we have tried to control bud infections in the field with single applications of Difolatan (5 qts/A), Bravo (6 pts/A), and lime sulfur (12 gal/A) applied each week from late Jan to mid-Feb, without success. On the average, about 3% of surface-sterilized symptomless buds were infected. There are several possible reasons for this, including bad timing and poor coverage. However last year we began to suspect that our test orchard, the most heavily infected in Ft. Valley, was less than ideal for this work. The inoculum load in this and most orchards with such poor sanitation is very high. At the onset, this feature seemed desirable. However, our orchard was so severely infected that we suspect systemic movement of the fungi is keeping pace with current tree growth.

This spring we compared infection in control plots with those sprayed with Difolatan on January 23rd. We isolated from 25 nodes in each of the four reps from our spray test, plating out 1" of twig beneath the fruit pedicel, the pedicel, and the developing fruit, along with bud scale remains, and the senescent calyx cup and anthers. On March 20, the twigs were more commonly infected than any other sampled tissue, except scales. Tween was added to sterilization fluid, and the individual scales were completely separated. The calyx cup and anthers, equally senescent by this date, contain little Botyrosphaeria by comparison. The only consistent significant difference resulting from the Difolatan spray was found in the total

parts infected, which is magnified by the effects of nodes with several infected parts. The data from each node plated out were kept separate, but no obvious trends appeared regarding the direction of growth of the fungus. The increasing fungus population over the spring months in these nodes could result from either bud infections, or systemic growth of the fungus.

A follow-up examination of trees sprayed in January 1983 showed no significant differences in fungus isolation between Difolatan (38% infected) and control (40% infected) twigs. Thus wood produced in the summer of 1982 was 39% infected by January 1984. This systemic movement is occurring much faster than I had previously thought, since branches do not usually exhibit symptoms until their third year. Isolations from new and 1 yr old wood in two unsprayed orchards corroborate this conclusion. Twigs plated out in this study were selected only if there was no canker nearby on the tree, and yet there is a very high percent infected (40%) by the time the wood is two years old.

Further evidence for systemic movement of the fungus was seen in greenhouse inoculations. Gum was extruded from the stems as much as 6 in above the inoculation point. When these stems were sectioned hyphae were found in the xylem vessels. From this, we conclude that there is no hope for bud swell sprays in a severely infected orchard.

Besides looking at the possible role of bud infections, we have isolated from cankers in the Ft. Valley area at approximately monthly intervals for the last two years. We take 5 twigs with cankers from each of four orchards, cut 1-in serial sections and plate them out on APDA. Most (40%) of the cankers contain all three species. We've found significant fluctuations in the levels of total Botryosphaeria, as well as changes in the species representation as the seasons change. In the summer, B. dothidea is the predominant species. In the winter and up until about March or April, B. obtusa predominates. Botryosphaeria rhodina grows most actively in the fall, but populations of this species are fortunately small. These fluctuations are probably due to differences in the cardinal temperatures for these species. The optimum for B. dothidea is 30C, (range 20-40C) (6), whereas the optimum for B. obtusa is 20C, (range 8-28C) (5).

If you consider the fungus isolated at the most distal point to the canker to inhabit the 'zone of colonization', and plot this against time, you see the same trends more clearly. Botryosphaeria obtusa is the major species in winter and spring, while B. dothidea is more frequently isolated at the margins in the summer. These data indicate that each species in turn is expanding the canker, and may explain why our trees do not outgrow infections, as almond trees do in California (4).

In collaboration with Jeff Daniell we have investigated the field resistance of Harken and Harbrite cultivars. He had made evaluations based on visual rating of external symptoms (3). We inoculated these trees and found that although there was significantly less vascular discoloration in Harken and Harbrite (55 and 58 mm, respectively), compared with the susceptible cultivar Winblo (84 mm), there was no difference in the spread of the fungus within the stems. Therefore this resistance

is probably related to host responses to infection rather than limitation of fungal growth.

Histological investigation of gummosis cankers reveal that hyphae are mostly intercellular, colonizing the cortex and ray parenchyma most often. The mycelium is most prevalent (or most visible) in the regions of ray dilation, possibly utilizing the crushed phloem as a readily available food source. Hyphae colonize rays in the xylem as well. The most noticeable effect of infection in the xylem is the formation of cavities through the disintegration of vessel elements beneath the canker. Vessel elements along the edges of cavities appear swollen, and may even loosen and appear to float in the viscous matrix within the cavity. I presume this substance to be gum, although it is not always golden in color. These cavities can be seen with the naked eye or a hand lens.

In some cankers, cracks may extend laterally along the cambium as well as radially to the outside surface. I think this depends on the nature of the original wound. Hyphae are very common along the cambium in these cases, and are then also likely to be found in a few vessel elements as well. More commonly, however, vessel elements are filled with gum, and occasional tyloses.

Phellogen formation is typical in these cankers, followed by sloughing of diseased tissue. This results in rough flakes of exfoliating bark which contribute to the characteristic external symptoms of gummosis cankers.

REFERENCES

1. Beisel, M. B. and F. F. Hendrix. 1984. Primary infection of apple buds by Botryosphaeria obtusa. Plant Disease 68: 707-709.

2. Britton, K. O. and F. F. Hendrix. 1982. Three species of Botryosphaeria cause peach gummosis in Georgia. Plant Disease 66: 1120-1121.

3. Daniell, J. W. and W. A. Chandler. 1982. Field resistance of peach cultivars to gummosis disease. Hortscience 17: 375-376.

4. English, H., J. R. Davis and J. E. DeVay. 1976. Relationship of Botryosphaeria dothidea and Hendersonula toruloidea to a canker disease of almond. Phytopathology 65: 114-122.

5. Foster, H. H. 1937. Studies of the pathogenicity of Physalospora obtusa. Phytopathology 27: 803-823.

6. Kohn, F. C., Jr. and F. F. Hendrix. 1982. Temperature, free moisture, and inoculum concentration effects on the incidence and development of white rot of apple. Phytopathology 72: 313-316.

7. Taylor, Jack. 1959. The distinctive nature of some apple disease conditions in Georgia. Pl. Dis. Rept. 43: 654-657.

8. Weaver, D. J. 1974. A gummosis disease of peach trees caused by Botryosphaeria dothidea. Phytopathology 64: 1429-1432.

GUMMOSIS OF PEACH TREES: CONTROL AND SUMMARY

F. F. Hendrix, Jr., and Kerry Britton
Department of Plant Pathology
University of Georgia, Athens 30602

Abstract

Infection of peach trees by Botryosphaeria dothidea, B. obtusa and B. rhodina occurs through lenticels (B. dothidea), wounds (all 3 species) and buds (B. obtusa). Fungicide sprays after pruning are a feasible means of preventing wound infection. Fungicide sprays at bud swell are being tested. Inoculum reduction aids in control. Increasing vigor of infected trees may allow them to recover.

In trying to control this disease, we started with heavily infected trees. This was a mistake, as we found that the fungus moved from infected wood to the current year's wood internally.

In trying to establish controls, we first tried to determine the inoculum courts, and time of infection. Besides wounds and lenticels, buds can serve as infection courts about the time they start growing. As with apples only B. obtusa is involved in this phase of infection (1,3). We think bud infection normally occurs at Fort Valley, Ga. the last week of January or the first week of February. We have attempted to control this infection by applying fungicides during this period, with limited success. We have had difficulty tying infection to a growth stage of the tree, and we think we applied the materials too late. The only promising material we have tested is Difolatan, because of its long residual. Then we discovered the movement of the fungus from old wood to new wood, and have abandoned spraying buds in infested orchards. We are starting over in newly planted trees.

We established a test in 1984 in cooperation with Walker Miller in which, on a new planting, we are applying Difolatan in January, after pruning and after harvest. All combinations of treatments are involved. It will be several years before data will be available, because of the long incubation period for gummosis.

Wounds are a major infection court. The most common wounds are pruning cuts. Research by Riley and Okie suggests that wounds are highly susceptible for about 3-7 days after pruning (2). Riley and Okie were able to reduce these infections with a single application of Difolatan.

Another infection point is lenticels. We are unsure as to the importance of this mode of infection. Hopefully, in the field, this will be of minor importance. The only approach to control of these infections that I can think of is to reduce the amount of inoculum.

The source of inoculum is primarily from dead wood in and under the trees. It has been shown that Botryosphaeria rots on apple can be partially controlled by removing this wood from the orchard, or by chopping it with a flail mower (4). We set up an experiment comparing infection in blocks in which the wood was flail mowed, versus bushhogging. We reduced the number of infected buds one year. This experiment was in a heavily infected orchard. Again, the discovery of movement from old to new wood caused us to abandon this experiment. We are re-establishing it in a new planting this winter.

Field observations suggest that vigorous trees can outgrow the fungus. To test this, as well as flail mowing, we are establishing plots at Byron this winter.

Treatments will be: discing, sod with a herbicide strip, sod plus herbicide plus fail mowing, sod plus herbicide plus irrigation, and sod plus herbicide plus increased fertilization. This experiment is in collaboration with Larry Pusey and Walker Miller. Data will not be available for several years.

Pusey and Riley have found suggestions that some cultivars may be resistant. A problem has been how to measure the amount of disease. Several cultivars were inoculated. Pusey and Riley rated disease based on the amount of gumming. Later we collected the specimens and determined the distance that the fungus had moved from the inoculation point, and the distance that internal browning extended. The data is currently being analyzed.

It is difficult to evaluate losses due to gummosis. I doubt that any meaningful evaluation can occur until we can control this disease, and have disease-free plots available. When loss estimates can be made, we will have to reassess the control measures in a cost-benefit analysis.

Gummosis certainly causes trees to have a terrible appearance, and it kills fruiting wood. I don't think it should cause panic when it appears. We have seen many orchards recover from the disease in a practical sense when vigor of the trees is increased, primarily by irrigation. Probably, the most sensible approach to the disease at this time is to increase vigor and reduce inoculum by flail mowing, or removing dead wood from the orchard. Hopefully, in the next 5 years, more definitive information on control will be available.

References

1. Beisel, Myra, F. F. Hendrix and T. E. Starkey. 1984. Natural inoculation of apple buds by Botryosphaeria obtusa. Phytopathology 74: 335-338.

2. Riley, C. C. and W. R. Okie. 1983. Peach tree pruning time in relation to susceptibility and spread of Botryosphaeria dothidea. Phytopathology 73: 798.

3. Smith, Myra and F. F. Hendrix, Jr. 1984. Primary infection of apple buds by Botryosphaeria obtusa. Plant Dis. 68: 707-709.

4. Starkey, T. E. and F. F. Hendrix. 1980. Reduction of substrate colonization by Botryosphaeria obtusa. Plant Dis. 64: 292-294.

ECOLOGY, CANKER AND CONTROL OF BROWN-ROT CAUSING MONILINIAS OF THE WORLD

L. R. Batra

Mycology Laboratory, Plant Protection Institute
U. S. Department of Agriculture, Agricultural Research Service
Beltsville Agricultural Research Center
Beltsville, Maryland 20705

Summary

Worldwide five species of Monilinia (Discomycetidae) cause brown rot cankers that are significant to stone fruits: M. fructicola (Winter) Honey, M. laxa (Aderhold and Ruhland) Honey, M. fructigena (Aderhold and Ruhland) Honey, M. mume (Hara) Yamamoto, and M. kusanoi (Takahashi) Yamamoto. Japan is the only country where all five species coexist. Apparently the species of Monilinia, host, and moisture content of the twigs and their environment determine whether or not stem cankers are formed from diseased blossoms or fruits. A total of 1100 lesions of M. fructicola on six peach cultivars was plated at four different occasions at Beltsville, MD: Within a day or two, "zero day", of complete decay of naturally infected fruits and thereafter at 15-20, 70, and 165 da. A succession of three significant fungi developed at the necrotic lesions. On "zero day", M. fructicola, Aureobasidium pullulans (DeBary) Arnold, and Valsa spp. were in the ratio of 1 : 0.46 : 0.04; at 15-20 da the ratios were 1 : 0.85 : 0.76; and at 70 day they were 1 : 0.16 : 1.62. After overwintering, i.e. at 165 da, the ratio dropped to 1 : 0.66 : 15, indicating that during the succession Valsa spp. took over M. fructicola and A. pullulans. Incipient cankers of M. fructicola on peach were not a cause of limb killing, and overwintered cankers did not produce conidia during its blossoming at Beltsville.

In many moist temperate regions, orchard crops and related ornamental of the genera Prunus, Malus, and Pyrus are attacked by several Monilinia spp., the brown rot cup fungi (Byrde and Willets 1977, Honey 1936, Wormald 1954). 'Brown rot canker' refers to cankers caused by these fungi (Jehle 1913). On a worldwide basis, these fungi are the most serious limiting factor in stone fruit production and their quality control. For the pathogen, brown rot cankers provide readily available food storage sites where the organism can aestivate or overwinter during unfavorable environmental conditions yet upon the return of good times it can produce primary or secondary inocula without much additional vegetative growth.

Brown Rot Fungi or Stone Fruit

The following general account concerns M. fructicola, M. laxa, and M. fructigena (for additional spp. see Table 1) for they cause similar infections on various plant parts; they differ from one another in their preference for hosts, and the severity of symptoms they produce.

The brown rot fungi are troublesome because (1) they produce abundant inocula on many related cultivated and wild hosts; (2) they infect buds, blossoms, shoots, young leaves, and fruits and they cause the familiar blossom blight, twig dieback,

Table 1. Monilinia spp. significant on stone fruits, host preference and geographic distribution, see figure 1 (climatic regions with sporiferous cankers in significant numbers are indicated by an asterisk)

M. fructicola (Winter) Honey	Peach, plum, sweet cherry, apricot; infrequent on apple, pear, and quince	North America, (* on P. nigra and in California on peach), Australia (* on peach), Japan (* on peach and apricot), Central and South America, South Africa, and Egypt
M. laxa (Aderhold and Ruhland) Honey	Apricot, sweet cherry, plum, peach, Japanese apricot (Prunus mume Sieb. and Zucc.); rarely pear and quince	Europe (* on Prunus crops) and N. America (* in California and Oregon, on apricot and almond)
M. fructigena (Aderhold and Ruhland) Honey	Apple, pear, and quince; apricot, peach, and other stone fruit	Europe (* on apple and rarely on prunus crops), European USSR (* on quince), Egypt, Morocco, Turkey, Israel, Iran, Korea, China, and Japan (* on apple)
M. mume (Hara) Yamamoto	Japanese apricot (Prunus mume) and apricots	Japan (* on Japanese apricots and apricots); the fungus is endemic to Japan
M. kusanoi (Takahashi) Yamamoto	Flowering cherries (several species) and sweet cherry	Japan (* on sweet cherry); the the fungus is endemic to Japan

cankers, and fruit decay; (3) they possess rather hardy overwintering structures that in some species can last for more than one year; and finally (4) their inocula are spread by wind, rain, insects, and birds, and by humans through unsanitary tools or containers.

Life Cycle

The life cycle of Monilinia has two stages and both are directly or indirectly relevant to canker control--the ascigerous, apothecial, or sexual stage, and the conidial or asexual stage (Fig. 2). In the spring, inocula from both stages cause primary infections in some localities.

Figure 1. Distribution map of Monilinia species causing brown rot of stone fruit.

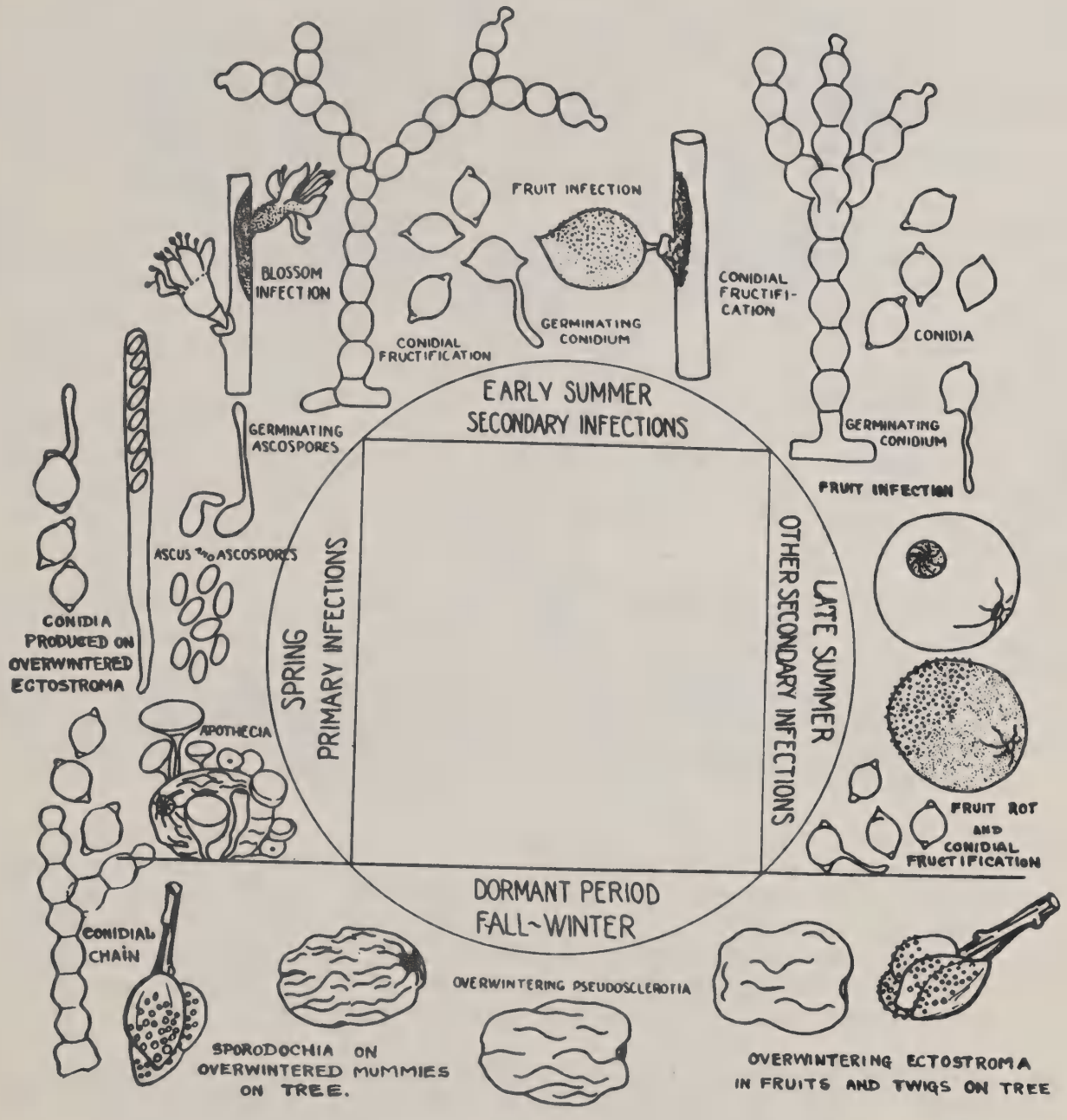


Figure 2. Diagrammatic representation of the life-history of Monilinia fructicola; conidia shown are somewhat atypical (slightly modified from Honey, 1936).

Apothecia

Pseudosclerotia in infected, mummified overwintered fruit that lay fully or partially buried in moist soil, or among vegetation, give rise to apothecia above the ground line. They forcibly eject ascospores into the air, which carry spores to susceptible host organs, usually blossoms in stone fruits, but in the endemic Japanese Monilinia kusanoi the infection court also is a nascent tender leaf or shoot. Blossom infection often results in stem cankers and successive crops of conidia are produced under wet conditions (Fig. 2, top center, heavily stippled area).

Conidia

Decayed fruit that remain attached to the tree through the winter, and any associated stem cankers present, also produce successive crops of conidia in the spring. The conidia are airborne and like ascospores, cause blossom blight. As in the ascosporic blossom infections, additional conidia are produced which now may attack any remaining blossoms or fruit.

Cankers and Their Significance

Etiologically speaking, cankers are fungus-filled, overwintering structures, analogous to mummies on the tree, or on the ground. All three can provide primary inocula for the blossom blight. Having caused blight in the blossoms or decay in the fruits, the fungus grows through the peduncle and into the twig and causes stem cankers, Fig. 2 (Jehle 1913, Landgraf and Zehr 1982, Roberts and Dunegan 1932, Sutton and Clayton 1972). Apparently the moisture content of the twigs and their environment determines whether cankers are formed from diseased blossoms or fruits (Weaver 1950, Willison 1936). Infrequently, a stem canker develops where rotten fruit abuts against a susceptible shoot. In stone fruits light colored gum, turning dark brown, often exudes at the bases of invaded flowers and fruit peduncles and twigs. Thus, depending on the species of Monilinia and host, and regional climatic conditions, brown rot cankers are important because:

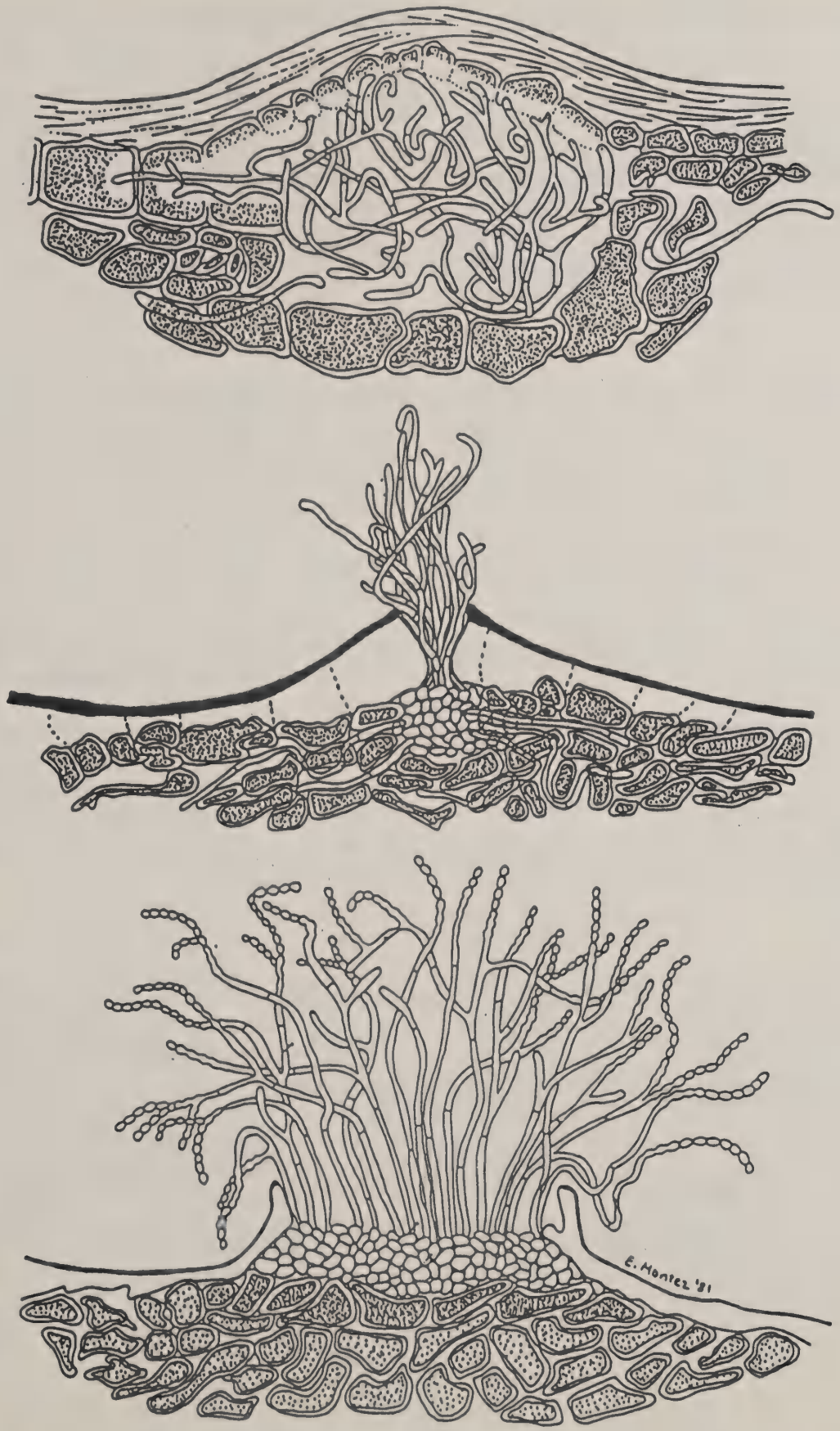
1. A portion of the fruit crop loss occurs as a result of the destruction of blossoms and fruit-bearing twigs and young fruit by the primary conidial inoculum from overwintered cankers. This is particularly significant for M. fructicola is parts of Australia and New Zealand where apothecia are unknown, or in California and Japan for M. laxa, a species where apothecia were collected only twice in a hundred years.

2. At harvest time the greatest damage occurs when under humid conditions the fungus may extend from the rotting fruit into twigs, killing them and thus reducing the crop for the following year. In the literature there are many examples of total loss of a peach or an apricot crop for the year following such an infection; an apricot orchard at Beltsville in the summer of 1978 was heavily damaged and the following spring nearly all of the tree died.

3. Incipient lesions by Monilinia spp. may be portals of entry for the harmful perennial successor fungi such as Valsa spp. (Willison 1936; Table 2).

Pathological Anatomy of Cankered Tissues

Monilinia-affected peduncles, twigs, and branches of Prunus crops, those of P. nigra, and perhaps other species show a characteristic brown discoloration in the cambium, followed by abundant formation of gum pockets in the same region. Roberts and Dunegan (1932) and Sutton and Clayton (1972) have described the pathological anatomy of peach infected by M. fructicola; similar anatomy of apricot infected by M. laxa is described by Zwygart (1970). In pomaceous fruit the mode of wound periderm formation is somewhat different and gummosis is subdued; otherwise the symptoms are about the same (Fig. 3-5).



FIGS. 3-5. An incipient, partially, and fully developed sporodochium of M. fructicola from an apple twig canker, respectively. Note stratified cuticle and fracturing of epidermal cells in FIG. 3, and fungus cells (unstippled) below conidiophores in FIGS. 4 and 5. Camera lucida drawings approx. X200.

Relative Frequency of M. fructicola in Cankers and Associated or Successor Fungi

The data on an ecological aspect of M. fructicola reported here are a portion of a monograph on Monilinia (Batra 1983). The work was conducted in the Beltsville orchards, where each of the 216 peach cultivars was being regularly examined for the occurrence of Monilinia spp. Over a period of 4 years (Batra 1979). The orchard received the following protective chemicals at concentrations comparable to commercial practice, and sulfur. During this period over 8000 isolations were made from necrotic sections for various experiments. Here I report on a fungal succession from six peach cultivars in 1975, one of the worst years for peaches, plums, and apricots.

Techniques

Within a day or two of complete decay, naturally infected fruit of peach cultivars Cullinan, Early Hale, Elberta, Fresno, Kimbo, and Royal Hale were identified on branches 1 to 3 cm in diam. Four series of isolations were made from the necrotic area. Only cankers (numbers in parenthesis) without a callus cover were used in the first three series but in the fourth series about half of the cankers appeared to have been partially covered by a callus: (i) the day the fruits were tagged (25); (ii) 15th through 20th day after tagging (100); and (iii) 70th day after tagging (50). The fourth series of isolations was from 50 mummies each of Elberta and Kimbo peaches that overwintered in the trees in February, 1976, before the trees bloomed. Fruits still attached to the pedicel and cankered wood were aseptically transported to the laboratory and the isolations made within 1-2 hr of their removal from the tree. The decayed fruit was removed and the pedicel with adjoining twig and branch was surface disinfested with 85% ethanol for 30 seconds, followed by dipping in 0.5% NaOCl (about 10% clorox) for 5 min. For each isolation, a potato dextrose agar plate received a section of nearly decorticated wood, approximately 8 x 8 x 5 mm^3, from immediately underneath the pedicel, and two similar sections about 1 cm laterally from it. Plates were incubated 3-8 da at room temperature and were periodically examined for fungi.

Observations

A total of 1100 lesions on six peach cultivars were plated at four different occasions and fungi were recovered from 46.5 percent of the lesions. Of the 150 lesions plated on the day of tagging, i.e. "zero day", M. fructicola, Aureobasidium pullulans, and Valsa spp. were in the ratio pf 1 : 0.46 : 0.04 (for percentages see Table 2). Alternaria and Rhizopus together were present in 8 percent of the isolations. The ratio (575 isolations) changes at 15-20 da for each cultivar and the incidence of both M. fructicola and A. pullulans decreased but Valso increased--1 : 0.85 : 0.76. At this time Alternari and Rhizopus were present in 13 percent of the isolations. Both these fungi are weak parasites, bordering saprophytism, but Valsa spp. commonly cause incipient cankers on peach (Wensley 1964). It is notable that Sutton and Clayton (1972) isolated M. fructicola in 88 percent of the lesions on branches below the fruit peduncle 30 da after artificially inoculating peaches in North Carolina. This survival rate is higher than ours (mean 27.5 percent) from the naturally infected material even though we isolated the fungus a few days earlier than they did.

Table 2. Percentage incidence of incipient Monilinia fructicola cankers on six cultivars of bearing peach trees with associated fungi, at Beltsville, MD, 1975-76

Cultivar	Monilinia fructicola			Aurebasidium pullulans			Valsa leucostoma [1]		
	Days After Tagging Lesions[2]								
	0	16-20	70	0	15-20	70	0	15-20	70
ELBERTA	40	25	4	16	24	0	0	10	24
FRESNO	32	40	18	24	24	4	0	18[1]	16
KIMBO	40	31	30	20	36	8	8	16[1]	26
EARLY HALE	48	12	0	28	15	0	0	24	10
ROYAL HALE	60	25	10	15	10	0	4	18	20
CULLINAN	72	32	12	32	32	0	0	40	24
	0	15-20	70 165[2]	0	15-20	70 165[2]	0	15-20	70 165[2]
TOTAL MEAN	48.6	27.5	12.33 3	22.5	23.5	2 2	2	21	20 45

[1] A total of six isolates on these dates were determined as V. cincta, according to Willison's (1936) descriptions.

[2] For the first five cultivars 25 necrotic lesions or associated cankers were used on the day of tagging; 100 cankers at 15-20 da; and 50 cankers at 70 da. For Cullinan only 25 cankers were used at each time; at 165 da 50 lesions each of only Elberta and Kimbo were available.

At 70 da (275 isolations) after the decay of fruit, Monilinia and A. pullulans had been overtaken by Valsa spp.--their ratio being 1 : 0.16 : 1.62. Also, at this time Alternaria and Rhizopus evidently were viable but not in sufficient numbers (together less than 3 percent) and Dothiorella spp. and Trichothecium roseum Link were also occasionally isolated. In February of 1976, at 165 da after tagging, relative frequency of Monilinia had dropped to 3 percent of the isolations versus Valsa spp., 45 percent (Monilinia : Aureobasidium : Valsa ratios being 1 : 0.66 : 15); other fungi were now less significant. This low viability for Monilinia agrees with similar data for other experiments at Beltsville but is again in strong contrast to 72 percent recovery of the fungus in North Carolina by Sutton and Clayton (1972) after overwintering. Roberts and Dunegan (1932) reviewed data by others on survival of M. fructicola in peach cankers following blossom blight, through winter in Georgia, Delaware, and Canada; the survival rate ranges from 0.0 to 75 percent of the cankers tested, depending on peach cultivar and location. Dunegan himself (in Roberts and Dunegan 1932) secured the fungus from 39 percent (n=105) of similar cankers in an experiment in Georgia in January and February, 1928, before the trees blossomed. However they summed up their findings as follows: "It has been the writers' experience that the fungus frequently does not live over in blighted twigs and cankers" (Roberts and Dunegan 1932 : 36). My data

on low survival of M. fructicola through the winter in cankers caused through decayed fruit (instead of through blossom blight!) at Beltsville confirms their general experience. Neither these authors nor I found conidia on overwintered peach cankers, although conidia are common during the season in which cankers are initiated. Fungus survival in cankers through the winter without the production of primary inoculum next spring is etiologically inconsequential.

Ecology and Control

In discussing the significance and control of brown rot cankers one must specify the pathogen and crop host species, the weedy wild hosts present in the area, and the regional climate. Due to limitations in the field we unfortunately neglect to attend to the insidous effect of primary inoculum on wild hosts of Monilinia spp.

In Monilinia laxa and M. fructigena, both of European origin, apothecia are very rarely found. The first species is established in the states and provinces along the Pacific in North America; it also occurs sporadically in Wisconsin, Michigan, and New York. In addition to mummies, characteristically M. laxa sporulates abundantly in cankers of many Prunus crops and ornamentals in its geographic range during wet periods in winter and spring (Hewitt and Leach 1939, Howard and Horne 1921). Thus in Europe, North America, Australia, and New Zealand, peach and other Prunus crops usually receive a dormant eradicant spray against M. laxa (Boesewinkel 1972, Byrde and Willets 1977, Wilson and Ogawa 1979). Monilinia fructigena prefers pomaceous genera and is uncommon on stone fruits.

In contrast to M. laxa, the native American M. fructicola of the predominantly eastern states produces apothecia commonly on peach, plum, and wild Prunus spp., e.g. P. nigra Ait., and P. angustifolia Marsh (Landgraf and Zehr 1982). Sporiferous cankers are relatively insignificant on peach but they occur on P. nigra. This species however in parts of Australia and California my require an eradicant spray on peach against sporiferous cankers and mummies (Kable 1983). In Japan all apothecia occur in the field (Harada and Batra, unpublished). All but the Japanese M. kusanoi form sporiferous cankers.

During the peach crop of 1975, an usually wet year, branches were killed 20 to 41 cm up and down from the decayed fruit of M. fructicola at Beltsville and many limbs gave the appearance of being scorched by fire. In late July 1978, during a separate outbreak, our apricot orchard 2 km away from peaches, was similarly devastated; during the fall and winter of that year many beautiful 8-20 yr old trees died and eventually we discontinued use of that orchard. This orchard also had several rows of plums, and included two mature trees of the cultivar Edwards. This cultivar is most susceptible to M. fructicola and is, therefore, seldom grown in the eastern United States. However, it thrives well in California, where M. fructicola is uncommon. It was my analysis that showed that the inoculum which killed the adjoining aprict trees largely came from these plums; the plums, over a period of 4 years, seldom bore healthy fruit but, instead, produced abundant mummies and sporiferous cankers.

Conclusions

1. Significance of the cankers depends on a brown rot fungus species, the crop and the regional climate. Cankers are generally known for most Monilinia species on their wild hosts, including those discussed here.

2. Incipient cankers of M. fructicola on peach were not a cause of limb killing in the Beltsville orchards but blighting of shoots is common. They were

relatively an insignificant source of inoculum during blossoming time. However, necrotic areas associated with this fungus evidently served as portals for the establishment of Valsa spp. Reviewing his 30 year research on peach brown rot control in 1949 Dr. Dunegan, the famed U.S.D.A. plant pathologist stated that "...in spite of the varied statements concerning the role of these cankers, no one has published definite data showing that the American brown rot fungus sporulates on the twigs the year following their production. We have followed this matter rather closely in the South and have stated that, although the fungus is alive in some of the cankers, the following spring, spores are not produced in sufficient number to be effective agents to the spread of the fungus (spores were noted on only on overwintered canker during the period 1921-1928)." He then concluded that there is no evidence that warrants recommending dormant spraying to control brown rot fungus.

3. Japan is the only country in the world where all brown rot Monilinias of stone fruit are found, occasionally in a single orchard. Cankers in all but one species produce significant quantities of inoculum on nearly all Prunus crops. Plant pathologists in each climatic region must ascertain for themselves as to the occurrence of sporiferous cankers of Monilinia and only then consider whether or not eradicative dormant sprays ar in order. What is a sauce for the goose may not be for the gander.

Acknowledgments

I thank Mrs. Marla Hunt for preparing the list of references cited, editorial comments, and technical assistance in the laboratory. Dr. Larry Douglass, statistician at Beltsville, reviewed data in Table 2.

Literature Cited

1. Batra, L. R. 1979. First authenticated North American record of Monilinia fructigena with notes on related species. Mycotaxon 8: 476-484.

2. Batra, L. R. 1983. Monilinia vaccinii-corymbosi (Sclerotiniaceae): its biology on blueberry and comparison with related species. Mycologia 75: 131-152.

3. Boesewinkel, H. J. 1972. Sclerotinia laxa, a new brown rot fungus in New Zealand. Orchardist New Zealand 45: 51-57.

4. Byrde, R. J. W., and H. J. Willets. 1977. The brown rot fungi of fruit: their biology and control. Pergamon Press, New York. xv + 171 p.

5. Dunegan, J. C. 1949. Peach brown rot control. Peach Annual Natl. Peach Council 1949: 26-29.

6. Harada, Y. 1977. Studies on the Japanese species of Monilinia (Sclerotiniaceae) (in Japanese, English summary). Bull. Fac. Agr. Hirosaki Univ. 27: 30-109.

7. Hewitt, W. B., and L. D. Leach. 1939. Brown-rot sclerotinias occurring in California and their distribution on stone fruits. Phytopathology 29:337-351.

8. Honey, E. E. 1936. North American species of Monilinia. I. Occurrence, grouping, and life-histories. Amer. J. Bot. 23: 100-106.

9. Howard, W. L., and W. T. Horne. 1921. Brown rot of apricots. Univ. California Agric. Exp. Sta. Bull. 326: 73-88.

10. Jehle, R. A. 1913. The brown rot canker of the peach. Phytopathology 3: 105-110.

11. Kable, P. F. 1983. New fungicides for suppression of sporulations by Monilinia fructicola from overwintering sites in peach trees in spring. J. Hort. Sci. 58: 45-50.

12. Landgraf, F. A., and E. I. Zehr. 1982. Inoculum sources for Monilinia fructicola in South Carolina peach orchards. Phytopathology 72: 185-190.

13. Roberts, J. W., and J. C. Dunegan. 1932. Peach brown rot. U. S. Dept. Agric. Tech. Bull. 328: 1-60.

14. Sutton, T. B., and C. N. Clayton. 1972. Role and survival of Monilinia fructicola in blighted peach peach branches. Phytopathology 62: 1369-1373.

15. Weaver, L. O. 1950. Effect of temperature and relative humidity on occurrence of blossom blight of stone fruits. Phytopathology 40: 1136-1153.

16. Wensley, R. N. 1964. Occurrence and pathogenicity of Valsa (Cytospora) species and other fungi associated with peach canker in southern Ontario. Canad. J. Bot. 42:841-857.

17. Willison, R. S. 1936. Peach canker investigations. II. Infection studies. Canad. J. Res. 14: 27-44.

18. Wilson, E. E., and J. M. Ogawa. 1979. Fungal, bacterial, and certain nonparasitic diseases of fruit and nut crops in California. Agric. Sci. Publications, Univ. California, Berkeley. 190 p.

19. Wormald, H. 1954. The brown rot diseases of fruit trees. Minist. Agric. and Fisheries (United Kingdon) Tech. Bull. 3: 1-113.

20. Zwygart, T. 1970. Untersuchungen über Wirt-:arasit-Beziehungen bei Moniliosen an Obstbäumen. Phytopathol. Z. 68: 97-130.

PYTHIACEOUS SPECIES ASSOCIATED WITH DECLINE OF CHERRY TREES IN MICHIGAN

M. L. Smither and A. L. Jones
Department of Botany and Plant Pathology
Michigan State University
East Lansing, MI 48824

Loss of cherry trees is a major problem in Montmorency sour cherry orchards of southwest Michigan, particularly on sites with heavy, poorly drained soils. The problem can be found in orchards of all ages, but the incidence of dead trees is highest when the trees come into bearing. Terminal growth is reduced for a year or more before the trees collapse. Root systems of dying trees exhibit extensive feeder root necrosis, and in advanced stages the larger roots and collar of the trunk are dead to the graft union. Affected trees are concentrated in low areas of the orchards except in severely affected orchards where dead and dying trees are distributed throughout the planting.

The objectives of this work were to isolate and identify the pythiaceous fungi associated with declining cherry trees and to determine the pathogenicity of these fungi to cherry.

Pythiaceous Fungi Isolated

Root samples were collected from dying trees in several orchards during the growing seasons of 1982, 1983, and 1984. Roots were soaked overnight in tap water and washed thoroughly before isolations were made on corn meal agar (CMA) or CMA amended with pimaricin-ampicillin-rifampicin-pentachloronitrobenzene (PARP) (1). *Pythium irregulare* was isolated approximately ten times more frequently than *Py. ultimum* and *Py. sylvaticum*. A few other *Pythium* species were also isolated. No *Phytophthora* species were isolated.

Soil samples were assayed for *Phytophthora* using the apple cotyledon baiting technique developed by Jeffers (2), except cherry leaf disks were sometimes substituted for apple cotyledons. Soil samples were sieved through 5 and 2 mm mesh sieves, and a 30 ml sub-sample was placed in a 250 ml beaker to air dry. After 3 days the soil in each beaker was moistened by adding 5 ml of sterile distilled water. The top of each beaker was covered with clear plastic held in place with a rubber band. After 3 days in a growth chamber at 21 C, the soil in each beaker was flooded with sterile distilled water to a depth of 2 cm above the soil surface. Apple cotyledons (variety McIntosh) and/or cherry leaf disks were floated on the surface of the water. Starting daily after the third day, any plant tissue with necrosis was removed and examined with a binocular microscope for *Phytophthora*. Isolations were made from infected tissue using PARP selective media. Identifications were made using the key of Waterhouse (7).

Ph. cactorum was isolated in low incidence from soil taken from six of the nine orchards (Table 1). The greatest number of isolations was from soil taken from orchard W. This site had previously been planted to apples, but the orchard was removed because Ph. cactorum, which was isolated from several trees (A. Jones, unpublished), had killed a high percentage of the trees. Ph. citricola was isolated from orchard G. in 1983. Among the five orchards where Phytopthora could be isolated, it was only isolated in both years from orchard J.

The incidence of Pythium in soil from several cherry orchards was determined with a soil assay. Four soil cores 2 cm in diameter and 15 cm in depth were taken from each of 10 trees per orchard between the drip line and the trunk. The soil cores were placed in plastic bags, transported to the laboratory in an ice chest, and stored at 4 C until processed.

The population of Pythium in each sample was enumerated using the surface soil dilution plate method (4). Soil samples were put through 5 and 1.68 mm mesh sieves. From each soil sample, 1 g of soil was removed for preparing 1:10, 1:50, and 1:100 dilutions in molten sterile 0.2% water agar in test tubes. Each suspension was mixed for 1 min with a Vortex tube mixer before diluting, and the diluted samples were mixed for 10 seconds before 1-ml subsamples were plated onto fresh PARP media. The subsamples were distributed over the surface of the media with a sterile glass rod. There were three replicate plates per dilution. The plates were stored in plastic bags in the dark at 21 C. After 42 hours the soil suspensions were removed by washing each plate under running tap water. A colony count was made and the number of propagules per gram of dried soil was determined.

Hyphal tips were transferred to fresh PARP plates from the edge of 5 to 15 fungal colonies obtained from each tree. After incubation for 48 hours at 21 C, hyphal tips were transferred to CMA. Fungal colonies were examined after 1 week and identified using the keys of Middleton (3) and Van Der Plaats-Niterink (6). Populations of Pythium in clay soils were between 33.2 and 437.0 propagules per gram of soil and in sandy soils they were 2.6 to 3.0 propagules per gram of soil (Table 2). The Pythium population consisted primarily of Py. irregulare, the heterothallic species Py. sylvaticum and Py. ultimum, and two unidentified species.

Pathogenicity Tests

Pathogenicity tests were carried out on 3-month-old and 6-week-old Mahaleb (Prunus mahaleb) seedlings in the glasshouse. Inoculum of Phytophthora and Pythium were prepared by growing each isolate in the dark at 21 C for 4-6 weeks in sterilized 500 ml flasks containing 200 ml Vermiculite moistened with 100 ml of a V8-juice solution (200 ml V8-juice, 2g $CaCO_3$, and 800 ml distilled water) (5). The inoculum was washed with distilled water over four layers of cheesecloth in a Buchner funnel to remove unassimilated nutrients, and mixed at the rate of 10 cc per 1000 cc of soil. The controls received Vermiculite and V8-juice but no fungus. Maximum daytime temperatures were 24 C with 14 hours of light. The nighttime minimum was 13 C. The 3-month-old but not the 6-week-old seedlings were flooded for 48 hours every 2 weeks. Dry weights of the shoots and roots were recorded after 2 months. The fungi were reisolated from root tissue using PARP media.

All the fungi caused a significant reduction in the dry weight of shoots and roots of the 3-month-old seedlings when compared to the control (Table 3). Ph. cactorum and Py. irregulare caused the greatest reduction in both shoot and root dry weight. Py. ultimum and Ph. megasperma caused the least reduction in dry weights.

All fungi caused a significant reduction in shoot length and shoot dry weights of 6-week-old mahaleb seedlings, and all except Py. sylvaticum and Ph. megasperma caused a significant reduction in root dry weights (Table 4). The greatest reduction in root dry weights was with Ph. citricola and Ph. cactorum, and Py. irregulare caused the greatest reduction in shoot dry weight.

Discussion

The association of Pythium species with declining trees in heavy soils and the pathogenicity of Phthium to seedlings indicates that these fungi are involved in cherry decline. The high incidence of Py. irregulare in cherry roots is significant and it is the most virulent of the Pythium species in pathogenicity tests. Pythium species have not been considered important in the decline of cherries because Phytophthora species have been shown to cause cherry decline in California (5).

It is difficult to define the role of Phytophthora species in cherry tree decline in Michigan because numerous isolation attempts made from 1981 to 1985 have yielded only small numbers of Phytophthora isolates from soil or directly from roots. The frequency of isolation is very low considering the large numbers of trees that are declining and the number of attempted isolations.

Literature Cited

1. Hayes, J. E., and Aldwinkle, H. S. 1982. Phytophthora root and crown rot of cherry in New York State. Stone Fruit Decline Workshop Proc. pp. 60-62 Michigan State University, E. Lansing, MI 48824.

2. Jeffers, S. M., and Aldwinckle, H. S. 1984. Baiting Phytophthora cactorum from naturally infested soil. Phytopathology 74:867 (Abstr.)

3. Middleton, J. T. 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club. 20:1-171.

4. Mircetich, S. M., and Kraft, J. M. 1973. Efficiency of various selective media in determining Pythium populations in soil. Mycopathol. Mycol. Appl. 50:151-161.

5. Mircetich, S. M., and Matheron, M. E. 1976. Phytophthora root and crown rot of cherry trees. Phytopathology 66:549-558.

6. Van der Plaats-Niterink, A. J. 1981. Monograph of the genus Pythium. Studies of Mycology No. 21 Centraalbureau voor Schimmelcultures, Baarn. 242 p.

7. Waterhouse, G. M. 1963. Key to the species Phytophthora de Bary. Mycol Pap. 92:1-22 Commonw. Mycol. Inst., Kew, Surrey England.

Table 1. *Phytophthora* species isolated from soil taken from around the roots of Montmorency cherry trees in southwestern Michigan that exhibited poor tree growth and decline.

Sampling period	Orchard	Number of samples	Number of isolates	
			Ph. cactorum	*Ph. citricola*
1983	B	43	4	0
	E	18	0	0
	G	23	4	3
	J	18	2	0
	M	18	0	0
	C	10	0	0
	L	38	0	0
1984	B	10	0	0
	G	10	0	0
	J	10	2	0
	C	10	1	0
	P	10	0	0
	W	10	8	0
	L	10	1	0
Total	-	318	22	3

Table 2. Populations of Pythium species isolated from clay and from sandy soils taken from Montmorency cherry orchards in southwest Michigan in 1984.

Orchard	Soil Type	Number of Pythium colonies[a]	Pythium species as percentage of total population					
			Py. irregulare	Py. sylvaticum	Py. ultimum	Py. sp. 1	Py. sp. 2	Py. spp.
B	Clay	76.1	69	8	13	10	-	1
G	Clay	124.6	42	14	21	4	19	-
J	Clay	219.0	24	56	9	6	5	-
C	Clay	55.7	47	48	-	-	5	-
L	Clay	54.3	60	23	6	11	-	-
P	Clay	74.7	64	1	22	11	-	2
W	Clay	33.2	8	23	44	25	-	-
G	Clay	76.7	73	7	9	3	8	-
U	Sand	2.6	-	38	-	38	24	-
N	Clay	437.0	17	36	12	12	33	-
Q	Sand	3.0	33	-	-	10	57	-

[a] Population expressed as the number of colony forming units per gram of dried soil. Values are the mean of ten soil samples per orchard. Each soil sample is the composite of four sub-samples per tree.

Table 3. Effect of various isolates of Phytophthora and Pythium on the growth of 3-month-old seedlings of Prunus mahaleb in infested soil.

Fungus	Isolate designation	Dry weights (g)[a]	
		Shoots	Roots
Ph. cactorum	B 1	5.7 a	1.2 a
Ph. cactorum	J 1	6.2 a	2.1 ab
Py. irregulare	J 213	7.7 b	1.8 ab
Py. irregulare	L 217	7.7 b	2.0 ab
Py. irregulare	L 208	8.1 b	1.9 ab
Py. irregulare	C 102	7.8 b	2.2 b
Ph. citricola	G 1	7.5 B	2.3 b
Ph. irregulare	G 1	8.2 bc	2.5 bc
Py. ultimum	B 17	8.1 b	2.8 bc
Ph. megasperma	G 3	9.2 c	2.5 bc
Control	California	10.2 c	3.4 cd
		10.9 d	4.0 d

[a] Values are means of six replicates. Values followed by different letters are significantly different (P=0.05) as determined by a Duncan's multiple range test.

Table 4. Effect of *Phytophthora* and *Pythium* on the growth of 6-week-old seedlings of *Prunus mhaleb* in infested soil.

Fungus	Shoot length	Dry weights (g)[a]	
		Shoots	Roots
Py. irregulare	26 a	2.1 a	1.2 b
Ph. citricola	26 a	2.3 b	1.0 a
Py. sylvaticum	26 a	2.3 b	1.4 c
Ph. megasperma	29 b	2.5 c	1.6 d
Py. sylvaticum	30 bc	2.5 c	1.4 c
Ph. cactorum	31 c	2.6 cd	1.0 a
Py. ultimulm	34 d	2.7 d	1.2 b
Control	38 e	3.3 e	1.5 c

[a]Values are means of eight replicates. Values followed by different letters are significantly different (P=0.05) as determined by a Duncan's multiple range test.

DEVELOPMENT OF PRUNING WOUND CANKERS IN ALMOND TREES CAUSED BY *PHYTOPHTHORA SYRINGAE*

M. A. Doster and R. M. Bostock. Graduate Research Assistant and Assistant Professor, respectively. Department of Plant Pathology, University of California, Davis, CA 95615.

ABSTRACT

Gumming cankers in mature almond trees associated with pruning wounds and caused by *Phytophthora syringae* were observed in the central valley of California in 1982-1984. In an almond variety trial in Butte County naturally infected pruning wound cankers were found in all twenty varieties observed and the mean cankers per tree were 0.41, 0.49, and 0.43 for the years 1982, 1983, and 1984 respectively. In two almond orchards in Colusa County in 1984 all the pruning wounds in 308 trees in one orchard and 485 trees in the other were surveyed for the association with a canker. The percentage of pruning wounds with cankers was 10.5% and 23.4% in the two orchards. The height and diameter of each pruning wound was measured. The percentage of pruning wounds with cankers increased significantly linearly ($P < .0001$, $r^2 = 0.969$) as the diameter of the pruning wound increased. Pruning wound cankers were observed at heights from 0.5 m to greater than 5 m. In one orchard there was a substantially higher percentage of pruning wounds with cankers at 2.0 m height than at 1.5 m and 1.0 m, whereas in the other orchard from 1.5 m to 3.5 m height the percentage was approximately the same. This was contrary to expectation if the inoculum was splashed up from the soil. The occurrence of pruning wound cankers was not random throughout the orchards.

Phytophthora syringae (Kleb.) Kleb. and other *Phytophthora* species are well known as pathogens causing root and crown rots of stone fruit trees (Mircetich, 1981, 1982; Mircetich et al., 1974). Since 1982 in California gumming cankers in mature, bearing almond trees (*Prunus dulcis* (Mill.) D.A. Webb) were frequently observed to be associated with pruning wounds made in fall and winter. *P. syringae* was consistently isolated from these pruning wound cankers and produced similar appearing cankers after pruning wounds of almond trees were inoculated with the fungus (Bostock and Doster, 1984a, 1984b). At the present time, evidence indicates *P. syringae* is the principal causal agent of pruning wound cankers in almond in California.

Some of the symptoms of pruning wound cankers are similar to those of crown and collar rot of almond caused by *P. syringae* and other *Phytophthora* species, but are associated with pruning wounds throughout the tree. The infection causes profuse sour-smelling gumming, leading to the appearance of large balls of gum on the bark (Fig. 1). When the outer bark covering an active canker is removed, irregular zonations of greenish-yellow and brown are observed. In old cankers the bark can appear sunken compared to nearby healthy tissue. The pruning wound cankers appear in winter soon after pruning, are active during spring, and seem to "die out" in summer. Occasionally a canker will girdle and kill a branch. Although we have been able to isolate *P. syringae* from cankers in April and May, all attempts to isolate in June have failed. For isolation from bark tissue a selective modified PV medium (Tsao and Ocana, 1969) was used. There are several good descriptions of

the morphological characteristics of P. syringae (Waterhouse and Waterston, 1964; Kouyeas and Chitzanidis, 1968).

The growth of P. syringae in host tissue and in media is very dependent on the temperature. Above 24 C there is no growth and the mycelium dies out (personal observation and Waterhouse and Waterston, 1964). However, at low temperatures P. syringae grows very well. In our experiments, at 2 C P. syringae grew well in cornmeal agar and was pathogenic in excised almond twigs. Cankers in excised almond twigs caused by P. syringae were larger than those caused by almond isolates of P. cactorum (Leb. and Cohn) Schroet. and P. citricola Sawada at temperatures less than 12 C but were smaller at temperatures greater than 12 C. These observations may explain why cankers in the field expand during winter and spring and die out in summer. The importance of rainfall to the severity of disease has not been determined, although presumably it is important. P. syringae fruit rot of apples was found to be associated with rain (Upstone and Gunn, 1978). The rainfall during fall and winter for the years 1982-1984 has been higher than normal in California. Climatic conditions in the central valley of California of cool, rainy winters (normal average temperature is about 7 C in Dec and Jan, 10 C in Feb, 11 C in March and Nov (extracted from Local Climatological Data of the NOAA)) seem very favorable for the development of pruning wound cankers by P. syringae.

There can be confusion of Phytophthora pruning wound cankers with other Phytophthora canker diseases of aerial parts observed occasionally. Sometimes Phytophthora cankers are observed in trunks and scaffolds where the canker has moved up from near soil level and really originated as a crown rot. Pruning wound cankers, on the other hand, will have a pruning cut near the center of the canker. Occasionally Phytophthora cankers will be associated with flooding of trees by overirrigation or a natural flood, allowing the zoospores to infect above soil level. Also, zoospores can be dispersed in contaminated water used during sprinkler irrigation leading to infection of aerial parts. However, pruning wound cankers occurred during winter when irrigation was suspended and in orchards where there had been no flooding.

From 1982 to 1984 an almond variety trial orchard of California State University of Chico, Butte County, CA, has been surveyed for the presence of naturally infected aerial cankers (Table 1). P. syringae was consistently isolated from these cankers. Cankers have been found on all twenty almond cultivars examined which included the common California cultivars Nonpareil, Mission, Merced, Ne Plus Ultra, Carmel, and Thompson. The average number of cankers per tree was about the same (approximately 0.4) for all three years (Table 1) indicating the severity of the disease was similar for all three years.

In order to determine the severity and pattern of development of the disease, two other bearing almond orchards were surveyed in spring, 1984 (Table 1). At the Nickels Estate Orchard, Colusa County, CA, in a block of 11 rows of 28 trees per row and in a commercial orchard, Colusa County, CA, for 485 trees, all the pruning wounds were examined for the association with a canker. The diameter and height of

each pruning wound was measured. P. syringae was isolated from pruning wound cankers in these orchards.
In general, a higher percentage of cankers was associated with larger pruning wounds. For the data from Nickels Estate Orchard (Table 2) a regression line, cankers per pruning wound = 0.019 + 0.0034 * diameter of pruning wound (mm), was significant ($P < .0001$, $r^2 = 0.969$). A multiple-infection transformation, which converts mean cankers per pruning wound to estimated infections per pruning wound, was used for the reasons given by Gregory (1948). This transformation gave a better fit ($r^2 = 0.971$) of the data to the regression line, infections per pruning wound = 0.015 + 0.0038 * diameter. In table 2, the infections per unit area and infections per unit circumference were estimated using the assumption that pruning wounds are approximately circular. The number of infections per unit circumference was approximately the same for all diameters of pruning wounds whereas the number of infections per unit area decreased substantially as diameter increased. This could occur if the fungal inoculum can only form cankers by infecting the inner bark around the outside of the pruning wound, but not the area of the exposed wood.

Cankers have been observed at heights from 0.5 m to greater than 5 m. In Nickels Estate Orchard the percentage of pruning cuts associated with cankers increased as the height increased, whereas in the commercial orchard the percentage was approximately equal for all heights (Table 3). This was unexpected because for other aerial diseases caused by P. syringae the disease is more prevalent closer to the soil. Fruit rot of apples caused by P. syringae occurred mostly on fruits within 50 cm of the soil (although infections have been observed at heights over 1 m) and rain splash from infested soil was believed to be the most likely means of dispersal (Upstone, 1978). Citrus brown rot caused by several Phytophthora species was usually found on fruit within 1 m of the ground and the zoospores of P. syringae were believed to be dispersed to the citrus fruit on the trees by wind blown rains (Fawcett, 1936; Feld et al, 1979). At this time, the data suggest that the inoculum inciting the pruning wound cankers was not dispersed from the soil by wind or rain, although the aerial dispersal of Phytophthora species that do not have caducous sporangia is poorly understood.

Pruning wound cankers were not randomly or evenly distributed throughout the orchards. The southern third of the orchard had 2.0%, 4.3%, 5.9%, and 5.6% pruning wounds with cankers for 2, 3, 4, and 5 cm diameter pruning wounds respectively, while the northern two-thirds had 25.4%, 25.0%, 35.0%, and 38.8% respectively. When the Nonpareil trees of the commercial orchard were divided into 9 approximately equal parts, a chi-square test was significant ($P < .001$) for the percentage of pruning wounds associated with cankers for each of the diameter classes. Among the Nonpareil in the Nickels Estate Orchard, there were parts of the orchard having very few cankers. No difference in cultural practice was associated with the distribution of cankers observed.

P. syringae has been found to cause diseases in almond, apricot, cherry, and peach trees. P. syringae caused crown rots of apricot, cherry, and peach trees in California (Mircetich, 1981), trunk cankers in almond trees in California

(Mircetich et al., 1974), a collar rot of one to four year old apricot, peach, and cherry trees in New Zealand (Smith, 1956), stem cankers in young apricot and peach trees in Oregon (Young and Milbrath, 1959), and collar rot of both young and mature almond and apricot trees in Greece (Kouyeas, 1971). Smith (1956) in inoculation experiments found apricot, peach, and cherry trees susceptible but plum resistant. Kouyeas (1971) found that the order from very susceptible to resistant was apricot, almond, peach, cherry, and plum trees. In our preliminary experiments when pruning wounds of mature trees were inoculated with an almond isolate of P. syringae, almond developed very large cankers, peach and apricot large cankers, and plum and prune very small cankers. P. syringae has been isolated from naturally occurring pruning wound cankers in apricot and French prune in California. Although other stone fruit trees besides almond are susceptible to P. syringae, the extent of natural occurring pruning wound cankers among the various stone fruits has not been investigated. It is possible that P. syringae causes diseases of stone fruit trees more frequently than commonly believed because of the difficulty in isolating from infected tissue.

LITERATURE CITED

Bostock, R. M. and Doster, M. A. 1984a. Association of Phytophthora syringae with pruning wound cankers in almond. Phytopathology 74:840 (Abstr.).

Bostock, R. M. and Doster, M. A. 1984b. Association of Phytophthora syringae with pruning wound cankers in almond trees. Plant Disease. Accepted for publication.

Fawcett, H. S. Citrus Diseases and their Control. McGraw Hill. New York. 1936. 656 pp.

Feld, S., Mange, J., and Pehrson, J. 1979. Brown rot of citrus: a review of the disease. Citrograph 64:101-106.

Gregory, P. H. 1948. The multiple-infection transformation. Ann. Appl. Biol. 35:412-417.

Kouyeas, H. 1971. On the apoplexy of stone fruit trees caused by Phytophthora spp. Ann. Inst. Phytopath. Benaki 10:163-170.

Kouyeas, H. and Chitzanidis, A. 1968. Notes on Greek species of Phytophthora. Ann. Inst. Phytopath. Benaki 8:175-192.

Mircetich, S. M. 1981. Phytophthora root and crown rot of deciduous fruit trees in California. Pages 42-48 in: Proc. XIII Ann. British Columbia Fruit Growers Assoc. Horticultural Forum, 17-18 November, 1981, Penticton, B.C. Canada.

Mircetich, S. M. 1982. Phytophthora root and crown rot of apricot trees. Acta Horticulturae 121:385-396.

Mircetich, S. M., Moller, W. J., and Chaney, D. H. 1974. Phytophthora crown rot and trunk canker of almond trees. Proc. Am. Phytopath. Soc. 1:58-59 (Abstr.).

Smith, H. C. 1956. Collar-rot of apricots, peaches, and cherries. Orchard. N.Z. 29:22-23.

Tsao, P. H. and Ocana, G. 1969. Selective isolation of species of Phytophthora from natural soils on an improved antibiotic medium. Nature (London) 223:636-638.

Upstone, M. E. 1978. Phytophthora syringae fruit rot of apples. Pl. Path. 27:24-30.

Upstone, M. E. and Gunn, E. 1978. Rainfall and the occurrence of Phytophthora syringae fruit rot of apples in Kent 1973-1975. Pl. Path. 27:30-35.

Waterhouse, G. M. and Waterston, J. M. 1964. Phytophthora syringae. In: C.M.I. Descriptions of Pathogenic Fungi and Bacteria. Commonwealth Mycological Institute Kew. No. 32.

Young, R. A. and Milbrath, J. A. 1959. A stem canker disease of fruit tree nursery stock caused by Phytophthora syringae. Phytopathology 49:114-115 (Abstr.).

Figure 1. Typical pruning wound canker caused by Phytophthora syringae showing large balls of gum on the bark around a pruning wound.

Table 1. Number of pruning wounds and trees surveyed and mean cankers per tree for three almond orchards.

Orchard	Year	Cultivar	Number of trees examined	Mean cankers per tree	Total pruning wounds	Percentage pruning wounds with cankers
CSUC Variety trial	1982	all 20	236	0.41	-----	-------
	1983	all 20	430	0.49	-----	-------
	1984	all 20	241	0.43	-----	-------
Nickels Estate Orchard	1984	Nonpareil	224	0.43	785	12.4
		Mission	56	0.09	119	4.2
		Ne Plus	28	0.14	105	3.8
		all 3	308	0.34	1009	10.5
Commercial orchard	1984	Nonpareil	313	0.88	1186	23.2
		Ne Plus	86	1.21	408	25.5
		Price	86	1.41	541	22.4
		all 3	485	1.03	2135	23.4

Table 2. Disease incidence for pruning wounds of various diameters in Nonpareil almond trees at Nickels Estate Orchard.

Diameter of pruning wound(mm)[b]	Pruning wounds with cankers(%)	Estimated infections[a] per pruning wound	per unit area(m^{-2})	per unit circumference(m^{-1})
10	4.8	0.050	631	1.58
15	6.8	0.071	402	1.51
20	10.0	0.105	335	1.68
25	9.7	0.102	207	1.30
30	11.8	0.126	178	1.34
35	13.9	0.150	156	1.36
40	15.3	0.166	132	1.32

[a]Multiple-infection transformation was used to convert cankers per pruning wound to infections per pruning wound.

[b]Only those diameter classes for which more than 50 pruning wounds were examined are listed.

Table 3. Percentage of pruning wounds associated with a canker at various heights in two orchards in Colusa County, CA.

Height of pruning wound (m)	Commercial Orchard Diameter of pruning wound (cm) 1.5-3.4	3.5-5.5	Nickels Estate Orchard Diameter of pruning wound (cm) 1.3-2.7	2.8-4.3
1.0	----[a]	----	4.8	7.7
1.5	20.0	26.4	8.1	12.9
2.0	17.1	25.2	18.9	18.5
2.5	24.3	29.6	---	---
3.0	13.6	31.3	---	---
3.5	----	34.8	---	---
All	19.1	28.4	9.2	13.2

[a]---- means fewer than 20 pruning wounds examined.

Peach Tree Decline in Relation to Peach Germplasm

Ralph Scorza
USDA-ARS, Appalachian Fruit Research Station
Kearneysville, WV 25430

A number of factors interact to reduce the longevity of peach trees as evidenced by the research presented in the proceedings of this workshop. Factors of primary importance in some growing areas, for example nematodes in the Southeast, may be of lesser importance in other areas. Cytospora canker, a major debilitating factor in the North, is not a significant problem in the South. Cultural practices, including pruning, weed, disease and pest control, and fertilization, may affect tree longevity in all peach production regions. An important element that should also be considered is the genetic variability of freestone peach cultivars now widely grown in the eastern U.S. and Canada.

The peach is native to China, and spread to Europe through the ancient trade routes. It was introduced into North America by the Spaniards early in the 16th century (3). Until the first half of the 19th century, most peach trees were grown from seed and the forces of natural selection were allowed to act on these genetically variable populations. Naturalized populations were adapted to particular environments on the North American continent. The 'Tennessee Naturals' occasionally used as rootstocks are remnants of such naturalized populations. As peaches began to be cultivated commercially, it is likely that only those trees with the most desirable fruit were used for planting new orchards; yet, since most trees were grown from seed, a great deal of genetic variability was maintained.

In 1850, 'Shanghai' or 'Chinese Cling' peach seedlings were introduced from Shanghai, China, to the U.S. Fruit quality of these genotypes was superior to most of the naturalized North American peaches and they were vegetatively propagated on a wide scale. When institutional breeding programs were initiated, 'Chinese Cling' and its descendants were intensively used as parents in the development of high quality cultivars. The 'Chinese Cling' germplasm pool has dominated freestone peach breeding programs in the U.S. and Canada for the past 100 years. With this germplasm, breeders have developed a succession of high quality, attractive, firm-fleshed cultivars which provide consumers across the continent with fruit throughout the summer months. Without these breeding efforts, the peach industry as we know it in the U.S., Canada, and throughout the world would not exist.

The cultivars 'Admiral Dewey', 'Elberta', 'Halehaven', 'J.H.Hale', 'Redhaven', 'Rio Oso Gem' and 'St. John' and its mutants (particularly 'South Haven'), and 'Southland' have been used most extensively as parents in the development of freestone peaches in the eastern U.S. (9). Of 30 cultivars selected generally on the basis of high production in the eastern U.S., 'J.H.Hale' is an ancestor of 22 and a parent of 5; 'Southland', an ancestor of 14 and a parent of 1; 'Halehaven', an ancestor of 12 and a parent of 6; 'Elberta', an ancestor of 9 and a parent of 2, and 'Redhaven' an ancestor of 5 and a parent of 2. The mean inbreeding coefficient for the 30 cultivars was .353 and values for individual cultivars ranged from .125 to .680 (9). Considering some common inbreeding coefficients: self-pollination - .500; parent offspring - .250; full sib - .250; half sibs - .125; and first cousins - .063; the inbreeding coefficients for these 30 widely grown cultivars is high. The ancestries of 'Elberta', 'Halehaven', 'J.H.Hale', and 'Southland' can be traced back to 'Chinese Cling' (Fig. 1). The 'Chinese Cling' seedlings imported into the U.S. originated from the southern group of Chinese peaches--a group adapted to warm moist summers but not considered cold- hardy in China (6). 'Elberta' ('Chinese

Cling' open-pollinated) and 'J.H.Hale' ('Elberta' self-pollinated) have been noted as having "cold tender" wood and flower buds, and these traits are transmitted to their progeny (1,2). They have also been found to be susceptible to Phytophthora collar rot (Phytophthora spp.), bacterial spot (Xanthomonas campestris pv. pruni), and Cytospora canker (Cytospora spp.) (1,5,8). Exceptional levels of resistance to low temperature injury or Cytospora do not appear to exist in contemporary cultivars (7). There are indications that present peach cultivars may be poor compartmentalizers of wounds and of wound invading organisms (10).

The lack of diversity in freestone peach germplasm negatively affects progress in breeding for disease and pest resistance from several points of view. First, if major genes for resistance do not exist in the germplasm pool, breeders cannot select for resistant genotypes unless natural or artificially induced mutations to resistance occur (4). Second, the development of procedures for selecting desirable genotypes is inhibited in the absence of a gradient from highly resistant to highly susceptible phenotypes which could be used to evaluate screening procedures. Small differences in susceptibility tend to be overridden by environmental effects (8). Third, without clearly defined resistant and susceptible phenotypes it becomes difficult to study resistance mechanisms.

Research on peach tree decline should not ignore the relatively narrow genetic background of U.S. freestone peach cultivars, particularly when testing variability between cultivars. The importation and critical testing of exotic germplasm with potential for disease, nematode, and low temperature resistance and the ability to efficiently compartmentalize injuries would be most useful (see Cain et al., this volume). The potential for developing peach and nectarine cultivars with significantly higher levels of cold hardiness and disease resistance does exist (9) and the stone fruit decline syndrome provides an impetus for development of genetically "tougher" trees.

Literature Cited

1. Blake, M. A. 1933. 'Elberta' and its selfed and chance seedlings lack hardiness. N. J. Agr. Exp. Sta. Cir. 287.

2. Blake, M. A. 1934. Additional facts in regard to the 'J.H.Hale' peach as a parent in breeding work. Proc. Amer. Soc. Hort. Sci. 30: 124-128.

3. Hedrick, U. P. 1917. The Peaches of New York. Rpt. N.Y. Agr. Exp. Sta. 1916.

4. Lapins, K. O. 1973. Induced mutations in fruit trees. In Induced Mutations In Vegetatively Propagated Plants. Proc. Panel on Mutation Breeding, International Atomic Energy Agency. Sept. 11-15, 1972. Vienna. pp. 1-19.

5. Leupschen, N. S. 1981. Criteria for determining peach variety susceptibility to Cytospora canker. Fruit Var. J. 35:137-140.

6. Li, Zailong. 1984. Peach germplasm and breeding in China. HortScience 19:348-351.

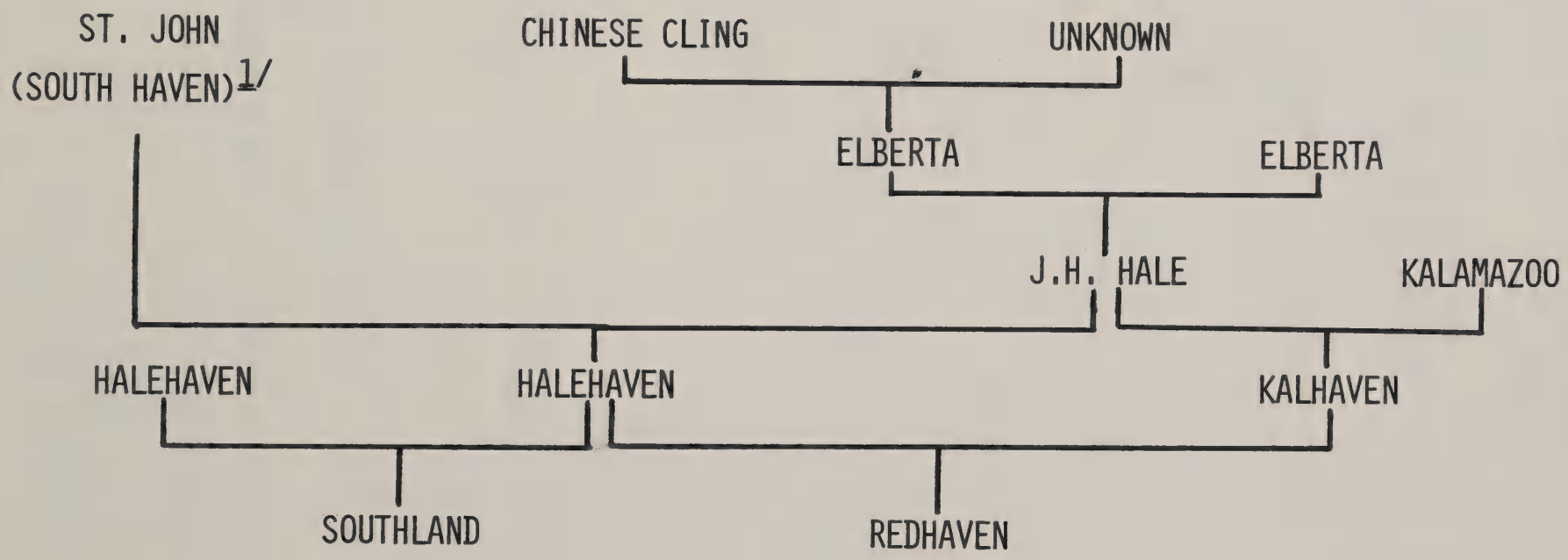
7. Mowry, J. B. 1974. Peach variety problems and breeding objectives in the Midwest. In: The Peach. (ed) N. F. Childers, The Communications Dept., Cook College, Rutgers University, New Brunswick, NJ. pp. 10-15.

8. Scorza, R. and P. L. Pusey. 1984. A wound-freeze technique for evaluating resistance to Cytospora leucostoma in young peach trees. Phytopathology 74:569-572.

9. Scorza, R., S. A. Mehlenbacher, and G. W. Lightner. 1985. Inbreeding and coancestry of freestone peach cultivars of the eastern United States and implications for peach germplasm and cultivar improvement. J. Amer. Soc. Hort. Sci. 110 (In press)

10. Shigo, A. L. and C. L. Wilson. 1982. Wounds in peach trees. Plant Dis. 66:895-897.

Fig. 1. Pedigrees of peach cultivars intensively used in the development current U.S. freestone peach cultivars.



1/ South Haven is a mutant of St. John

A Conceptual Model of Stone Fruit Tree Decline

M. W. Brown
West Virginia University
Appalachian Fruit Research Station
Kearneysville, West Virginia

The problem of stone fruit tree decline, as has been presented at this workshop, is complex and a solution can only be arrived at through the interaction among many disciplines. Agricultural engineers, pathologists, epidemiologists, physiologists, horticulturists, nematologists, entomologists, and others must not only work on the individual aspects of decline within their realm but also interact with other disciplines so that the pieces of the puzzle can be integrated into a unified picture. To aid in viewing the whole picture, a model is often of use. Models can be useful in several ways: one can see how each piece of information fits into the overall picture, one can more easily see the multiplicity of interactions and how each component is interdependent, it can reveal gaps in knowledge, and possibly reveal strategies which may lead to healthier trees. The conceptual model I present in this paper was developed from the information presented in this workshop. I hope that it will help both in organizing the information presented and in directing future research.

Stone fruit tree decline, simply stated, is that an apparently healthy tree dies through a series of episodes with one or more of a group of causal agents. How this comes about, the causal agents, and other aspects of the problem have been studied in detail by the people at this workshop. But the problem is not just the canker, nematode, improper pruning, insect, etc.; it is a sequence of events gradually leading to the total collapse of the entire system, death. Such a sequence of events includes not only repeated injury and attack by pathogenic organisms but also physiological, chemical, and morphological responses by the host. Death is as much due to depletion of the tree's reserves, through repeated responses to injury, as it is to pathogenic infestations.

The conceptual model of this process is presented in Figure 1. Throughout the discussion of the model there are two important concepts to keep in mind. First, the system is an ecological system. There are populations of different species with their own population dynamics and interspecific interactions; the whole system is a community with a structure and succession of its own. Second, stone fruit tree decline is dynamic. There is a continual response of both the tree and pathogens to each other and changing conditions.

There are several mediating factors which affect all of the components and interactions of the model. These factors are important in either influencing the severity of the pathogenic attack or the tree's susceptibility. Some of these are stress (e.g., foliar infestation by pathogens, root infestations, gummosis), genetic weakness of a cultivar, individual genetic differences within a cultivar, time of year, nutrition, condition and number of previous wounds, weather, site and soil factors, and any other factor that can influence overall vigor of the tree. Some of these mediating factors are constant throughout the decline (genetic, site), while others are dynamic and can be affected by the decline process itself.

The first event which initiates stone fruit tree decline is that a healthy tree becomes physically injured, or there is some other kind of breakdown in the tree's defense. Some of the more common injuring mechanisms are improper pruning, freezing, mechanized harvesting, and feeding by vertebrates or insects. Injury does two things to the tree. First, it breaks through the protective layer (bark)

exposing more susceptible tissues. Second, it initiates the tree's wound response system. Breakdown of the tree's defense system may be a result of systemic infection, stress, or some unknown agent or condition.

Once an injury occurs, two processes begin simultaneously: the tree responds to wounding, and pathogenic organisms begin to colonize the wound. It is in this component of the model, which is repeated again and again, that the dynamic aspect of decline is important. Neither the attacking organisms nor the tree's responses are independent. Each constantly responds to the conditions of the other. An ecological succession also takes place among the attacking organisms within the wound. There is an initial colonization by pioneer type organisms, exploitation of the resource, and the maturation of a community of organisms within the wound. The mature community includes several trophic levels including organisms antagonistic to, and predatory (parasitic) on the pathogens. This is one area of possible research into manipulation of the community for controlling decline.

Some of the pathogenic organisms attacking the wound are viruses, sub-viral pathogens, bacteria, fungi, nematodes, and insects. Host responses include gum ducts, phenols, callus tissue, and various forms of compartmentalization. An important fact to keep in mind with regards to the tree's responses is that they require the use of energy, which must be diverted from other uses such as fruit, growth, maintenance, or storage. Each successive injury depletes the tree's energy reserve further, thereby increasing stress.

At this point, the decline syndrome can go in one of two pathways. The barrier zone established by the tree to wall off infection may be incomplete or the barrier zone may be penetrated by the pathogens. In either case, infection spreads out from the wound re-initiating the interactions between tree and pathogens discussed previously. This is one area where the lesser peachtree borer may be important. The borer feeding in the wound may penetrate through the barrier zone, creating an avenue of infection for other pathogenic organisms. The borer could also circumvent the barrier zone, being attracted by volatiles in the wound, the borer may create a secondary wound beyond the barrier zone. Other mechanisms that also may help in penetrating the barrier zone are frost cracking, improper surgery on the wound, mechanical harvesting, or very aggressive growth of the pathogen.

The second pathway for decline to follow is that the tree successfully contains the infection, but is less vigorous due to the energy consumed in containment. The tree may completely recover, but more likely it will be re-injured, thus starting the whole cycle over again, with less energy reserve after each episode.

The processes of continued injury, recovery, and re-injury, or the pathogens breaking through the barrier zone are not mutually exclusive. At any point a wound that has been incompletely walled off may eventually be successful in containing the infestation. On the other hand, a tree which has successfully walled off repeated wounds may not be successful at a future wound. Also, the same process, at various stages of development, takes place at many sites on the tree simultaneously. In any case, if stone fruit tree decline proceeds through its typical scenario, the tree is eventually weakened to the point where its responses are inadequate and the tree dies as a result of a lack of energy to perpetuate its essential life processes.

This model is how I see the stone fruit tree decline problem as it has been discussed at this workshop. It is my hope that the model will be of use in organizing the diverse information presented. The model may also be of use as an overview of stone fruit tree decline, revealing how each portion of the syndrome is

interrelated with the rest. I feel that a model such as the one presented is important in directing further work on the separate components and their interactions. It is important to have an overview such as this so that the information can be integrated and the problem of stone fruit tree decline can be examined as a whole.

This work was completed under a cooperative agreement between the U.S.D.A. Agricultural Research Service and West Virginia University.

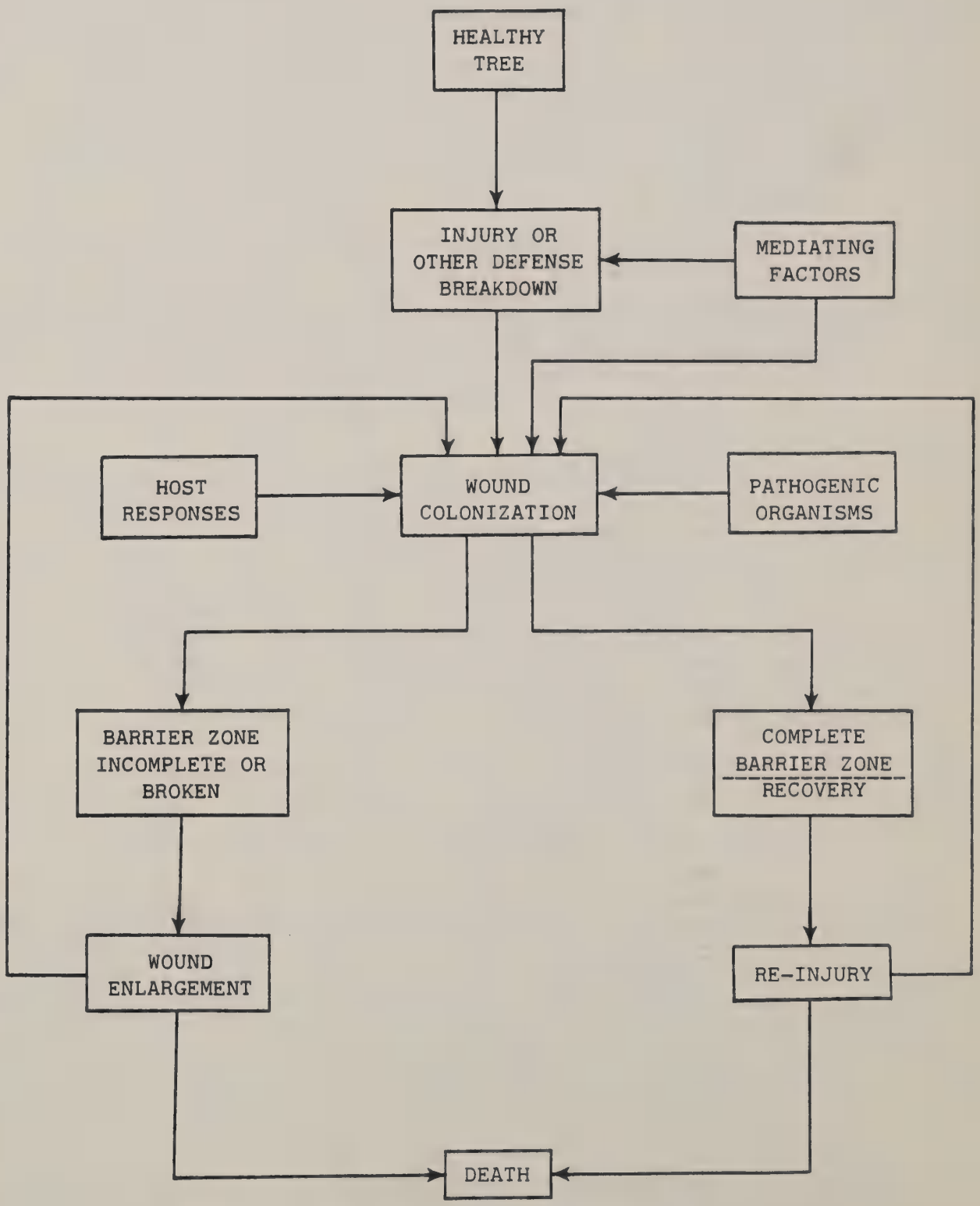


Figure 1. Conceptual model of stone fruit tree decline, see text for details.

PARTICIPANTS

1984 Stone Fruit Tree Decline Workshop

HERB ALDWINCKLE, Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva, NY 14446. (315/787-2011)

ED ASHWORTH, USDA-ARS, Appalachian Fruit Research Station, Route, 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

JOSEPH BARRAT, West Virginia University Experiment Farm, Box 303, Kearneysville, WV 25430. (304/876-6353)

LEKH BATRA, USDA-ARS-NER, Mycology Laboratory, Bldg. 011A, BARC-West, Beltsville, MD 20705. (301/344-2317)

MICHAEL BARNEY, Office of IPM Programs, 11 AG Hall, Michigan State University, East Lansing, MI 48821. (517/355-1855)

ALAN BIGGS, Agricultural Canada Research Station, Vineland Station, Ontario, Canada LOR 2E0. (416/562-4113)

WALTER BITTERLIN, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456. (315/787-2011)

STEVEN BLIZZARD, West Virginia University Experiment Farm, P.O. Box 303, Kearneysville, WV 25430. (304/876-6353)

DEBORAH BRETH, Penn State Fruit Research Lab, P.O. Box 309, Biglerville, PA 17307. (814/865-4700)

KERRY BRITTON, University of Georgia, Athens, GA 30602. (404/542-3030)

MARK BROWN, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

GALEN BROWN, Agriculture Engineering, Michigan State University, East Lansing, MI 48824. (517/355-1855)

BILL BUTT, USDA-ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

DAVID W. CAIN, Department of Horticulture, Clemson University, Clemson, SC 29631. (803/656-3311)

BURT CARGILL, Agriculture Engineering, Michigan State University, East Lansing, MI 48824. (517/355-1855)

ROBERT CLINE, Horticultural Research Institute of Ontario, Vineland Station, Ontario, Canada LOR 2E0. (416/562-4141)

DANIEL COOLEY, Department of Plant Pathology, University of Massachusetts, Amherst, MA 01003. (413/545-0111)

JOHN CORDTS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

MARK DOSTER, Department of Plant Pathology, University of California, Davis, California 95616. (916/737-2011)

BILL DOWLER, PDRL, USDA, ARS, Ft. Detrick, P.O. Box 1209, Bldg. 1301, Frederick, MD 21707. (301/663-7344)

ELKIE ENDERT, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607. (919/737-2011)

HAROLD FOGLE, 2014 Forestdale Drive, Silver Springs, MD 20903. (301/439-7676)

JERRY FRANKLIN, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

GEORGE GREENE, Department of Horticulture, Pennsylvania State University, P.O. Box 309, Biglerville, PA 17307. (814/865-4700)

LENARD GILREATH, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430 (304/725-3451)

FREDDI HAMMERSCHLAG, USDA, ARS, NER, Bldg. 011A, Room 130, BARC-West, Beltsville, MD 25430. (301/344-2752)

FLOYD HENDRIX, Department of Plant Pathology, University of Georgia, Athens, GA 30602. (404/542-3030)

KENNETH HICKEY, Pennsylvania State University, Box 309, Biglerville, PA 17307. (814/865-4700)

BILL HORTON, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

AMY IZZEONI, Department of Horticulture, Michigan State University, East Lansing, MI 48824. (517/355-1855)

BRUCE JAFFEE, Pennsylvania State Fruit Research Station, P.O. Box 309, Biglerville, PA 17307. (814/865-4700)

WOJCIECH JANISIEWICZ, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

ALAN JONES, Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824. (517/355-1855)

ED KLOS, Department of Plant Pathology, Michigan State University, East Lansing, MI 48824. (517/355-1855)

ARCHIE LATHUM, Auburn University, Department of Botany, Plant Pathology and Microbiology, Auburn Alabama 36830. (205/826-4000)

STEVE MILLER, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

JOHN MIRCETICH, Department of Plant Pathology, University of California, Davis, CA 95616. (916/752-1919)

DICK OKIE, USDA, ARS, SE Fruit & Tree Nut Research Lab, P.O. Box 87, Byron, GA 31008. (912/956-5656)

BRIAN OTTO, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

RICHARD OWINGS, Clemson University, Clemson, SC 29631. (803/656-3311)

LEE PARISH, USDA, ARS, Fruit Research Laboratory, 1104 Wenatchee Avenue, Wenatchee, WA 98801. (509/390-0317)

DONALD PETERSON, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

CAROL L. PINNELL-ALISON, SDS Biotech Corporation, 4712 Edwards Mill Road, Raleigh, NC 27612

LARRY PUSEY, USDA, ARS, SE Fruit & Tree Nut Lab, P.O. Box 87, Byron, GA 31008. (912/956-5656)

CHARLES C. REILLY, USDA, ARS, SE Fruit & Tree Nut Research Lab, P.O. Box 87, Byron, GA 31008. (912/956-5656)

DAVID RITCHIE, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607. (919/737-2011)

LAURE RITTENBURG-KENYON, SDS Biotech Corporation 3401 Foxboro #G, Woodridge, IL 60517. (312/983-1489)

DAVID ROSENBERGER, New York State Agricultural Research Station, P.O. Box 727, Highland, NY 12528. (315/787-2011)

RALPH SCORZA, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

ALEX SHIGO, U.S. Forest Service, University of New Hampshire, Durham, NH 03824. (603/862-1234)

MARGARET SMITHER, Department of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824. (517/355-1855)

JOHN SPRINGER, Department of Plant Pathology, Rutgers, The State Univ. of NJ, New Brunswick, NJ 08903. (201/932-1766)

JAMES TRAVIS, The Pennsylvania State University, University Park, PA 16802. (814/865-4700)

JERRY UYEMOTO, Department of Plant Pathology, University of California, Davis, California 95616. (916/752-0107)

TOM VAN DER ZWET, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

EUGENE VARNEY, Department of Plant Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903. (201/932-9375)

HOWARD WATERWORTH, USDA, ARS, NPS, Room 228, Building 005, BARC-West, Beltsville, MD 20705. (301/344-3915)

JOHN WELLS, USDA, ARS, Hort Crops Quality Research, Rutgers University, Cook College, P.O, Box 231, Building 6021, New Brunswick, NJ 08903. (201/932-9881)

BILL WELKER, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-34510

WAYNE WILCOX, Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva, NY 14456. (315/787-2011)

ROBERT WILLIAMS, WV Department of Agriculture, Plant Pest Control Division, Capitol Building, Charleston, WV 15305. (304/348-2212)

CHARLES WILSON, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

MIKE WISNIEWSKI, USDA, ARS, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430. (304/725-3451)

KEITH YODER, Winchester Fruit Research Lab, PO Valley Avenue, Winchester, VA 22601. (703/667-8330)

ROGER YOUNG, West Virginia University Experiment Farm, Box 305, Kearneysville, WV 25430. (304/725-3451)

ELDON ZEHR, Department of Plant Pathology, Clemson University, Clemson, SC 29631. (803/656-3311)

☆U.S. GOVERNMENT PRINTING OFFICE: 1985-618-488-40325

55